The Journal of Laboratory and Clinical Medicine

VOL. X ST. LOUIS, MO., AUGUST, 1925 No. 11

CLINICAL AND EXPERIMENTAL

PERNICIOUS ANEMIA*

BY JOHN W. SHUMAN, M.D., F.A.C.P., LOS ANGELES, CALIFORNIA

HERETOFORE all anemias, including those presenting a pernicious anemic picture, were labeled secondary when a definite cause was found. In the anemia in which no cause was found, and presenting the pernicious anemic picture, the diagnosis of a primary anemia always held. Pernicious anemia as a diagnostic entity should be relegated.

Considering pernicious anemia from all angles it is difficult to accept any view other than that it is the result of a *defense reaction* of the body, as a whole, against toxemia; a long continued fight finally resulting in a state of chronic invalidism, leading to death. Its cardinal symptoms are grave anemia, hemosiderosis, general weakness, moderate loss of weight, tachycardia, fever, gastrointestinal, visceral, and cord changes. Its course is marked by exacerbations and remissions.

Reviewing recent literature, the facts stand out clearly that:

1. The cause is not understood.
2. Its recognition is not early enough.
3. Its symptomatology is not rationally explained.
4. There is no logical linking of its cause and course.
5. Its treatment is still symptomatic and empirical.

ETIOLOGY AND PATHOGENESIS

William Hunter[1] considered "long standing oral and gastric sepsis" to be the cause of pernicious anemia. Sepsis is a cause but there are other contributing factors; for example, environment, pregnancy, cancer, endocrine dis-

*Presented before the Santa Monica Branch of the Los Angeles County Medical Society, held November 18, 1924, at the Soldiers Home Hospital, Sawtelle, California.
From Department of Internal Medicine, Soldiers Home Hospital, Sawtelle, California.
Received for publication, January 12, 1925.

turbance, etc., which act as accessory causes, in an individual in which chronic sepsis exists.

The first stage is a long continued disease, *a toxemia*. The toxemia is due to a bacteriolysis destroying the invading organism and liberating the toxic molecule which is then free to combine with the body cell (for example, the cord cell). Bacteriolysis, cytolysis, and hemolysis belong to the third order of Ehrlich's "side-chain-theory."

The second stage is a high grade hemolysis formation without a correspondingly high antihemolysis formation resulting in,

The third stage, a hemolysis which is demonstrated by the anemia, the excessive urobilin excretion, the hemosiderosis, and the blood picture.

The fourth stage is a disturbance of the hematopoietic system, a primary stimulation, shown by bone marrow hyperplasia and an increase in the reticulated cells.

These four stages are the prepernicious anemic stages during which hemolysis gradually occurs, usually escaping recognition because the hematopoietic system is compensating. This prepernicious anemic stage should be suspected if there is an excess of urobilin[2] in the urine, stools, and duodenal contents, and an increase of reticulated cells. Prior to and during this stage "preventive medicine" is most important:

1. A search for and removal of toxemic agents.

2. A correcting of faulty habits of living, and removing contributing factors, if possible, thereby keeping the health index high.

The fifth stage is exhaustion of the hematopoietic system as demonstrated by reversion to the embryonic type of red blood cell formation. During this stage, the condition is usually diagnosed as "pernicious anemia."

That a toxemia exists is demonstrated by the anemia, fever, malaise, tachycardia, weakness, increased metabolism, enlarged thyroid, brain, cord, gastrointestinal, and visceral changes. That it is a chronic toxemia is also shown by the leucopenia, lymphocytosis, loss of weight, exacerbation, and fatty degeneration of the heart.

It may be noted here that pernicious anemia sufferers seem remarkably free from intercurrent infections, and usually succumb to the disease itself. This shows a high grade immunity on the part of the individual.

The toxemia precedes the anemia, for often the brain and cord symptoms manifest themselves before the blood picture does.

Concerning the spinal cord changes, they occur in 75 per cent of all cases at some time, usually early but frequently overlooked because they are not searched for. Weakness of the arms and legs should be considered sufficient grounds for minute study. They are not necessarily of permanent nature because they are frequently recovered from during a remission. The severity of the cord symptoms is not closely associated with the degree of blood picture and vice versa. They are usually manifested by signs of tabes dorsalis or a spastic paralysis or a combination of the two.

The sympathetic nervous system plays a dominant rôle in the etiology of these symptoms, by central stimulation due to toxemia, with a peripheral expression. Achlorhydria, by many writers treated as a cause of pernicious

anemia, can be explained by a long continued sympathetic stimulation causing inhibition of the gastric secretion, not only hydrochloric, for there is also an inhibition of intestinal secretions and motility causing atonic constipation and periodic diarrhea.

Gastrointestinal tract changes are those encountered in toxemia, viz., gingivitis, stomatitis, glossitis, gastritis, enterocolitis, and proctitis. These are most marked during exacerbations. They may precede and usually improve during a remission.

The visceral changes, enlarged spleen, and liver, occur in all cases. Albuminuria is frequent.

The hypertrophy of the spleen is due to overactivity making good in the rôle it was created for, viz., ridding the blood stream of debris; in this instance a malicious thing, for it destroys the immature type of red blood cell,—the only blood cell the patient has: hence splenectomy has been popularized because it extends the patient's life for a few more years in the majority of instances.

COURSE

Each exacerbation leaves the patient in a more precarious state. Finally, exhaustion of the hematopoietic system, as is witnessed in that individual who completes the course, takes place. There is no doubt but that many die from the acute toxemia and are never permitted to reach the stage of bone marrow exhaustion; while others recover without reaching this stage; leaving a third class, necessarily the minority, to develop the grave anemia.

In pernicious anemia, the basal metabolic rate is usually increased, showing that the oxygen carrying capacity of the hemoglobin is not lessened. The lung ventilation remains the same. The total amount of hemoglobin is absolutely lessened due to a diminished cell count and diminished blood volume. This is partially compensated for by tachycardia and malaise. Exertion causes pallor, shortness of breath, vertigo, and excessive tachycardia.

For discussion, three case records are submitted, the one in the abstract, for he is dead, the other two are still with us.

CASE 1.—Demonstrates the following symptoms: (1) early nervous; (2) gastrointestinal; (3) blood system changes; (4) visceral evidence of hemolysis; (5) progress to fatal termination.

No. 4325, W. S., age thirty-four, admitted to the service of W. C. Allison, M.D., Feb. 15, 1924, complained chiefly of "pain in the upper abdomen." His father died from, "pernicious anemia (?)." The patient's sister stated that he had a "number of attacks of mental disturbance (amnesia), following his discharge from the service, June, 1919"; in 1920, he had a definite attack of persistent diarrhea; a gradual failing in health for the last one and one-half years with his color becoming more sallow and yellowish, accompanied by upper abdominal pain. One year prior to his admission here, he had an attack of abdominal colic, jaundice, and clay colored stools.

Physical examination.—Height, 70 inches; weight, 150 pounds (usually 165); color, sallow; mentally, apathetic; heart, soft systolic murmur at base; tender gall bladder area; spleen, palpable; internal hemorrhoids; cord changes were evidenced by rectal and urinary bladder incontinence, painful cramps and weakness of lower limbs, and absent knee-jerks.

Laboratory findings.—Urine, specific gravity, 1.026; hyaline, fine, and coarse granular casts; numerous pus cells and an occasional red blood cell. Blood, red blood cells, 1,912,000; hemoglobin, 40 per cent; color index, one-plus; blood platelets, 104,000; following transfusion, red blood cells, 2,300,000; white blood cells, 5,000; hemoglobin, 58 per cent; color index, one-plus; further transfusions, and on May 1, red blood cells, 2,072,000; white blood cells, 5,200; hemoglobin, 52 per cent; color index, 1.37; marked delle, poikilocytosis

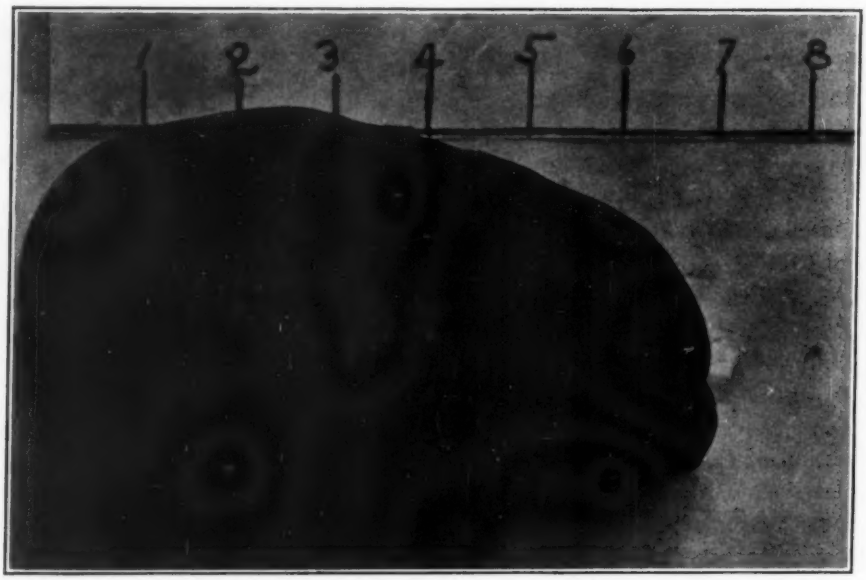

Fig. 1.

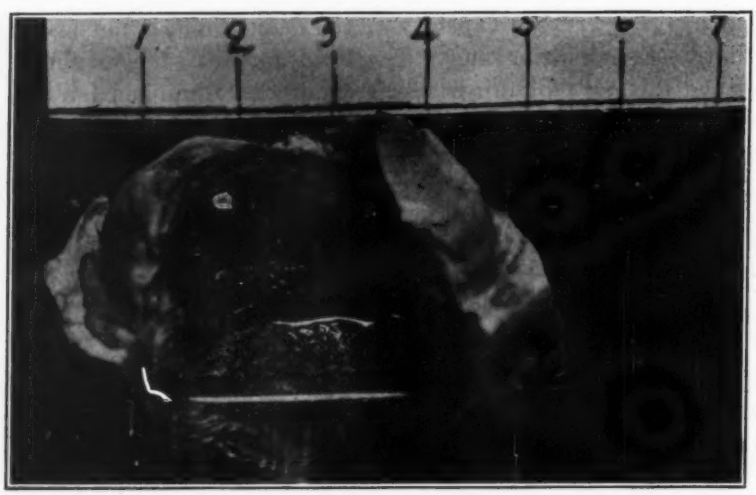

Fig. 2.

and anisocytosis; Wassermann, negative. Gastric analysis, gastrointestinal x-ray series, and spinal puncture were not done on account of patient's lack of cooperation.

A working diagnosis of "pernicious anemia, due to chronic toxemia," was made. The gall bladder, at operation, by James A. Mattison, M.D., was found to be, "definitely and chronically infected"; it was drained. His progress notes from then on to September 6, when he died, show, "progress from bad to worse."

Autopsy by W. C. Allison, M.D., revealed: "(1) old adhesions between gall bladder and duodenum; (2) a greatly thickened pylorus; (3) chronic appendicitis; (4) hypertrophic cirrhosis of liver; (5) splenomegalia [See Fig. 1]; (6) fibrotic pancreatitis; (7) diverticula of urinary bladder." [See Fig. 2.]

It is regretted that permission for a complete autopsy was not obtained.

CASE 2.—Demonstrates: (1) cord; (2) gastrointestinal; (3) blood; and (4) visceral changes.

No. 6081, F. A. F., age forty-five years, admitted to service of G. S. Craig, M.D., Sept. 20, 1924, complained of "General weakness, especially in legs, and gastric disturbance."

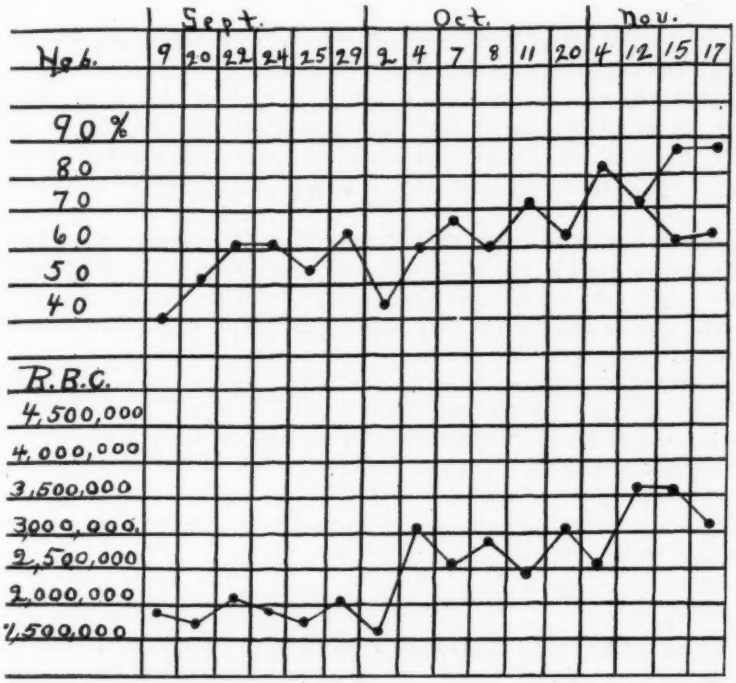

Fig. 3.

CASE 2. September 9. No nucleated red cells or myelocytes; marked anisocytosis and poikilocytosis.
September 22. Autohemic therapy.
September 26. Autohemic therapy.
October 4. Autohemic therapy.
October 11. Slight anisocytosis and poikilocytosis. Slight central depression of R. B. C.
November 12. No pathologic white cells found; polymorphonuclears predominating type of cell; no nucleated reds. Cells staining more uniformly, and showing very little poikilocytosis and anisocytosis.
November 13. Autohemic therapy.
November 15. No pathologic white cells; slight anisocytosis and poikilocytosis. Red cells uniformly stained.

His father died at age of sixty-three, from "uremia." He had "typhoid-malaria and yellow fever in 1899"; in January, 1924, a hemorrhoidectomy was performed.

Physical examination.—Undernourished, feeble man, with marked pallor; thyroid, one-plus; exophthalmus, slight; slight lagging of upper lids; slight wrinkling of forehead; the backs of the neck and hands *markedly* pigmented, this has been present since the "yellow fever"; hair, scant; six lower remaining teeth in fair condition (pyorrhea cause for loss); nose, right septal spur; tonsils, chronically infected; right inguinal hernia; still has protruding and bleeding hemorrhoids, a spastic sphincter, and hypersensitive rectum; a slight varicocele on the left; marked weakness and numbness in both legs and feet; a marked Rhomberg; gait, ataxic; absent knee jerks; tender lumbar muscles.

Laboratory findings.—Red blood cells, 1,710,000; white blood cells, 4,600; hemoglobin, 52 per cent; color index, one-plus; marked aniso- and poikilocytosis; Wassermann, negative; refuses spinal puncture; stomach, hydrochloric deficit of 37, combined acids 9; urine, a few pus cells; x-ray findings by R. C. Shawhan, M.D., reports, "evidence of duodenal ulcer; irregular hypomotility; and chronic colitis."

A working diagnosis of "pernicious anemia due to chronic toxemia," was made. His focal infection has been removed. Rest, autohemic and hydrochloric therapy seem to have improved him clinically for a remission is establishing itself (Fig. 3). Blood count Nov. 15, 1924, red blood cells, 3,780,000; white blood cells, 3,600; hemoglobin, 80 per cent; color index, one-plus; blood platelets, 310,000; poikilocytosis and anisocytosis, slight; cells stain uniformly.

CASE No. 3.—No. 5814, L. W., age thirty-seven, admitted to the service of W. C. Allison, M.D., June 26, 1924, complained of "weakness."

Father died from "kindey disease"; mother from "cancer of womb"; patient operated upon in 1919, in France, for rectal fistula and hospitalized for five months following, for "weakness." There seems to have been a remission, for it is not until December 1923, that the next hospital record appears; when it was discovered that he had but 500,000 red blood cells.

Physical examination.—Height, 68 inches; weight, 133 pounds; a marked lemon-tint pallor of the skin; slight but general lymph gland enlargement; hearing, defective; tonsils, diseased; nose, right deflected septum; gums, spongy and bleed easily; two apical abscesses of teeth; heart, systolic murmur at base; tender gall bladder area; a stricture of urethra; fissure of rectum.

Laboratory findings.—Urine, faint trace of bile, indican, and a moderate number of pus cells present. Blood, red blood cells, 560,000; white blood cells, 3,500; hemoglobin, 30 per cent; color index, 2.6; blood platelets, 104,000; coagulation time, seven minutes forty-five seconds; Wassermann, negative; bilirubin index of blood serum, 40 (normal 10 to 15); fragility test, normal; stomach, total acidity 10, with hydrochloric deficit of 18.

X-ray series of gastrointestinal tract by R. C. Shawhan, M.D.: "gastroptosis, irregular motility, caecal ptosis, and colonic angulation."

SPINAL FLUID ANALYSIS

Progress notes.—September 16, tonsils removed by Arthur E. Gill, M.D., cultured and hemolytic streptococci grew. Frequent gastrointestinal disturbances have occurred (vomiting, diarrhea, and abdominal pain), although dilute hydrochloric acid 10 to 20 drops t. i. d. seems to control. Frequent transfusions of 450 c.c. of blood from various donors have been given with apparent beneficial results. Blood findings Nov. 16, red blood cells, 2,300,000; hemoglobin, 81 per cent; color index, 1.76; aniso-and poikilocytosis, slight; repeated counts show the white blood cells never over 4,600, and never below 3,500 with the decrease in polymorphonuclear cells constant.

In Cases 2 and 3 all focal infection has been eradicated; rest has been maintained; transfusion and autohematization have been performed as often as we thought wise and here we are with a remission evidently taking place.

TREATMENT

Although a chapter in itself, it is briefed here:

1. Rest: this cannot be overstressed for obvious reasons; it lessens katabolism and damage to the vital organs, especially the heart.

2. The detection and removal of all focal infection:

(a) chronic upper respiratory (sinus, etc.) infection;

(b) chronic oral (gum, tooth, tonsil, tongue, etc.) infection;

(c) gastrointestinal and accessory visceral (stomach, gall bladder, pancreas, small and large gut, appendix, rectum, etc.) infection;

(d) genitourinary and reproductive tract, infection.

3. Hemictherapy:

(a) Transfusion: often gives a supply of a number of red blood cells equal to two-thirds of the patient's total count. These cells have been shown to survive a sufficient length of time to warrant this form of treatment; it may lessen hemolysis. Much care should be exercised in the selection of a donor in this class of patients, picking one that is free from not only acute but chronic infection, syphilis not among the greatest.

(b) Autohemic therapy for the production of antihemolysis with the advantage that it is easy to procure and in some instances the results have not hindered a remission.

5. Arsenic, diet, fresh air, and sunshine. Arsenic probably lessens hemolysis by depressing the endocrine system.

Diet should be nutritious and easily digestible; and dilute hydrochloric acid gtts. q.s. t.i.d. p.c. to supply the deficiency.

6. Splenectomy should be done:

(a) after all focal infection has been eradicated;

(b) after transfusing has lessened hemolysis; and

(c) during a remission when the blood is in a fair condition.

Splenectomy eradicates an important red cell destroying agent, thus preserving the immature cells, the best the patient can produce.

Regarding autopsy in "pernicious anemia," it is an old story to hear the remark, "Autopsy findings clearly demonstrated pernicious anemia." I offer the suggestion that that is what the pathologist set out to see, the effect rather than the cause. Thoroughgoing autopsies like physical examinations are few and far between. Few autopsies embrace a search for focal infection or a study of the endocrine glands. The fault lies at the clinician's door, for he lacks interest, not only in securing autopsy material but failure in witnessing and assisting the pathologist in his search.

An early recognition is insisted upon; grave or severe anemia, instead of "pernicious," as a diagnostic entity should be used, for these patients have learned that our medical forefathers and most of our brothers diagnose and treat pernicious anemia as "hopeless."

SUMMARY

1. Pernicious anemia has a cause, a long standing toxemia.

2. This toxemia is usually due to bacteria, causing a high grade hemolysis, which exhausts the blood-forming system.

3. It is the duty of the physician to accept this dictum to find and remove, if possible, cause or causes, the first step in treatment.

4. Following or failure of No. 3, the treatment is symptomatic.

REFERENCES

[1]Hunter, William: Severest Anemias, New York, 1909, Macmillan Co.
[2]Stitt: Bacteriology, Blood Work, Parasitology, Philadelphia, 1923, P. Blakiston's Son and Co., p. 677.

THE ACTION OF NITRITES UPON PULMONARY CIRCULATION*

I. NITROGLYCERIN

BY GEORGE R. LOVE, M.D., AND HUGH MCGUIGAN, M.D., CHICAGO, ILL.

THE present communication deals with the effect of nitroglycerin upon pulmonary arterial pressure and excised vessels, and attempts to explain the apparent contradiction in the effects on the pulmonary and other vessels. Macht[1, 2] in his studies of the action of various drugs upon excised pulmonary arteries found that the nitrite group caused a contraction of these vessels. This action is unusual in view of the commonly accepted statement that the nitrites are smooth muscle depressants.

Bradford and Dean,[3] Wood,[4, 5] and Petitjean[6] observed that the nitrites increased pulmonary arterial pressure. Bradford and Dean attributed the mechanism to "back pressure" from defective cardiac action. Wood and Petitjean believed that nitrites produced active constriction of the pulmonary vessels, and the latter substantiated his belief by observing a blanching of the lungs, particularly evident following amyl nitrite.

The method used for excised pulmonary arteries has been described by Macht.[1, 2] Dogs were used in the present investigation, including the experiments with excised vessels. For anesthesia several methods were used: ether first, then decerebration; ether first, then urethane; urethane alone. No difference was observed in the anesthetics except through their influence upon central vagal and vasomotor tone. Pulmonary pressure was recorded by the methods described by Francois-Frank[7] and Jackson,[8] but chiefly by a water manometer. The arterial cannula was inserted either into the left pulmonary artery or its branch to the upper lobe. Carotid pressure was recorded by the usual mercury manometer. Experiments were made with open and closed chests, but chiefly upon the former.

Several brands of nitroglycerin tablets were purchased upon the open market. The tablets were dissolved in 1-3 c.c. of saline and injected into a femoral vein. The dosage varied from 0.0006 to 0.006 gm.

Nitrolycerin in doses of 0.0006-0.002 gm. usually caused a transient rise of pulmonary pressure varying from 1 to 60 mm. H_2O. The average elevation was about 25 mm. H_2O in the open chest. (See Fig. 1.†) If the pulmonary pressure was normally high (320-500 mm. H_2O), nitroglycerin lowered it as well as carotid pressure. (See Fig. 3.)

That the rise of pulmonary pressure following nitroglycerin is due to active constriction of the pulmonary vessels is, however, questionable. Indeed,

*From the Department of Pharmacology and Therapeutics, University of Illinois, College of Medicine, Chicago, Illinois.
Received for publication, October 9, 1924.

†The only original tracings obtainable for reproduction were lost after leaving the author's possession.—Publisher.

it seems probable that constriction is not concerned in the action. The chief factors in causing the elevation of pulmonary pressure are: (1) an increase of heart rate due to the effect of lowered systemic blood pressure upon vagal tone, and (2) a transfer of blood from the arterial to the venous system; thus an increase of minute output. For (1) the rise of pulmonary pressure follows from three to ten heart beats after the carotid pressure begins to fall. (2) The height of the pulmonary elevation corresponds to about the low level of the carotid pressure. (3) The pulmonary and carotid pressure curves are roughly diametrically opposed in direction. (4) When cardiac action is impaired by larger doses such as 0.003-0.006 gm., the pulmonary pressure usually falls. (5) The stroke of the ventricular volume curve (as determined by the rubber dam cardiometer) is more complete, and the volume of the heart is diminished during the rise of pulmonary pressure. (6) The increase of pulmonary pressure is dependent to a large extent upon the increase of heart rate. For (a) the increase of rate is more effective if the heart rate was normally slow (below 100). (b) Cutting the vagi when in good tone causes a rise of both carotid and pulmonary pressures. (c) If the medulla is depressed by anesthesia so the heart rate is 180-210, nitroglycerin may cause a very slight rise, no effect or even a fall of pulmonary pressure, whereas the carotid falls distinctly. (d) When the vagi, which have been functioning effectively, are severed, the elevations of pulmonary pressure upon subsequent injections of nitroglycerin are usually diminished and sometimes abolished. (7) In a hypodynamic circulation with vagi not functioning (long anesthesia and exposure or after repeated doses), nitroglycerin produces a fall of pulmonary as well as carotid pressure, whereas epinephrin is still effective in elevating both.

If the pulmonary pressure is normally high, nitroglycerin even in doses as small as 0.5 mg. lowers pulmonary as well as carotid pressures. (See Fig. 3.) Thus it appears that when the pulmonary vessels are under a certain tension, nitroglycerin reduces the resistance in the pulmonary as well as the systemic circuit.

If, with a high pulmonary pressure which falls after nitroglycerin, pituitrin be given, the pulmonary pressure falls much farther from a reduction of cardiac output. If nitroglycerin is now repeated, while the carotid pressure is still high and the pulmonary pressure is low, pulmonary pressure rises temporarily. (See Figs. 3 and 4.)

The importance of the transfer of blood from arterial to the venous system in increasing pulmonary pressure may be seen by connecting the femoral artery to the femoral vein by a cannula. When the arterial flow is released, the pulmonary pressure rises promptly the first or second beat after the carotid begins to fall. The rise of pulmonary pressure is greater in proportion to the fall of carotid than that which occurs after nitroglycerin. (See Fig. 2.)

Nitroglycerin does not stimulate the excised pulmonary arterial rings or strips. This is true also when the tissue is suspended in its own defibrinated blood and the nitroglycerin is dissolved in blood. The doses employed varied from 0.0006 gm. to 0.0084 gm. with the tissue suspended in a 30 c.c. container.

This method for testing excised pulmonary arteries is inadequate to demonstrate depressant action. Thus papaverin, benzyl acetate and benzyl alcohol, as well as nitroglycerin, failed to relax these vessels. The failure to relax is probably due to the fact that the nonmuscular tissues are able to maintain the lifting load. The depressant or toxic action of nitroglycerin can be demonstrated by comparing two tissue suspensions. If one preparation receives nitroglycerin first, it responds very feebly or not at all to subsequent applications of epinephrin or barium, whereas the untreated pulmonary strip contracts vigorously to these drugs.

SUMMARY

Nitroglycerin in small doses often produces a rise of pulmonary pressure due to increased cardiac output from acceleration and increased venous return. Nitroglycerin reduces a high pulmonary pressure apparently by dilating the pulmonary arteries. It does not stimulate excised pulmonary arteries of dogs. Thus the action of nitroglycerin is the same upon pulmonary and systemic arteries, although less marked upon the pulmonary vessels. The action upon pulmonary pressure is, however, often counterbalanced or overbalanced by the secondary effects of its action upon the systemic vessels. The action of nitroglycerin on pulmonary circulation does not justify its administration in the treatment of pulmonary hemorrhage (cf. 1, 2). Experimentally, at least, it appears to be contraindicated in hemoptysis.

REFERENCES

[1]Macht: Jour. Pharm. and Exper. Therap., 1914, vi, 13.
[2]Macht: Jour. Am. Med. Assn., 1914, lxii, 524.
[3]Bradford and Dean: Jour. Physiol., 1894, xvi, 37.
[4]Wood: Am. Jour. Physiol., 1901-1902, vi, 283.
[5]Wood: Jour. Exper. Med., 1911, xiv, 326.
[6]Petitjean: Jour. de physiol. et de path gén., 1908, x, 403.
[7]Francois-Frank: Arch. de Physiol., 1895, p. 744-816.
[8]Jackson and Raap: JOUR. LAB. AND CLIN. MED., 1920, vi, 1.
[9]Wiggers: Am. Jour. Med. Sc., April, 1911.

THE ACTION OF NITRITES UPON PULMONARY CIRCULATION

II. Sodium and Amyl Nitrite*

By George R. Love, M.D., Hugh McGuigan, M.D., and
Charles E. Wiley, B.S., Chicago, Illinois

BRADFORD and Dean,[1] Wood,[2, 3] and Petitjean[4] observed that the nitrites increased pulmonary arterial pressure. The former investigators attributed the mechanism to "back pressure" from defective cardiac action, whereas Wood and Petitjean believed that nitrites produced active constriction of the pulmonary vessels. Macht[5, 6] reported that the nitrite group (sodium and amyl nitrite, nitroglycerin, and erythrol tetranitrate) stimulated the excised pulmonary arteries of the pig, ox, and human.

Love and McGuigan[7] in a recent communication demonstrated that nitroglycerin under certain conditions produced a rise of pulmonary pressure, due to a greater minute output from cardiac acceleration and increased venous return. If the pulmonary pressure was normally high, nitroglycerin lowered pulmonary as well as carotid pressure. We concluded that nitroglycerin has the same depressing action, though less marked, upon the pulmonary arteries as upon the systemic arteries. However, the effects of this action of nitroglycerin upon the pulmonary arteries are often counterbalanced or overbalanced upon pulmonary pressure by the secondary effects of its action upon the systemic vessels.

All experiments were performed upon dogs. The technic employed in this investigation was described in the previous communication.[7]

PULMONARY ARTERIAL PRESSURE

Amyl Nitrite.—The preparation was administered by breaking a five minim pearl in the rubber tubing connecting the tracheal cannula and Woulfe's bottle. The pulmonary pressure in some animals rose from 1 to 60 mm. H_2O following amyl nitrite, while in others the pulmonary pressure fell with the carotid pressure. The rise of pulmonary pressure is not produced by active constriction, but is apparently caused by a greater minute output from cardiac acceleration and increased venous return. Thus, the actions of amyl nitrite and nitroglycerin upon pulmonary circulation are the same, and the arguments presented in the previous paper on nitroglycerin are applicable to amyl nitrite.

Thus, in brief, with a combination of a high carotid, a low or moderate pulmonary pressure, good vagal tone, and a sudden drop of carotid pressure following amyl nitrite or nitroglycerin, the pulmonary pressure rises; whereas, a low carotid, a high or moderate pulmonary pressure, poor vagal tone, and

*From the Department of Pharmacology and Therapeutics University of Illinois, College of Medicine.
Received for publication, November 12, 1924.

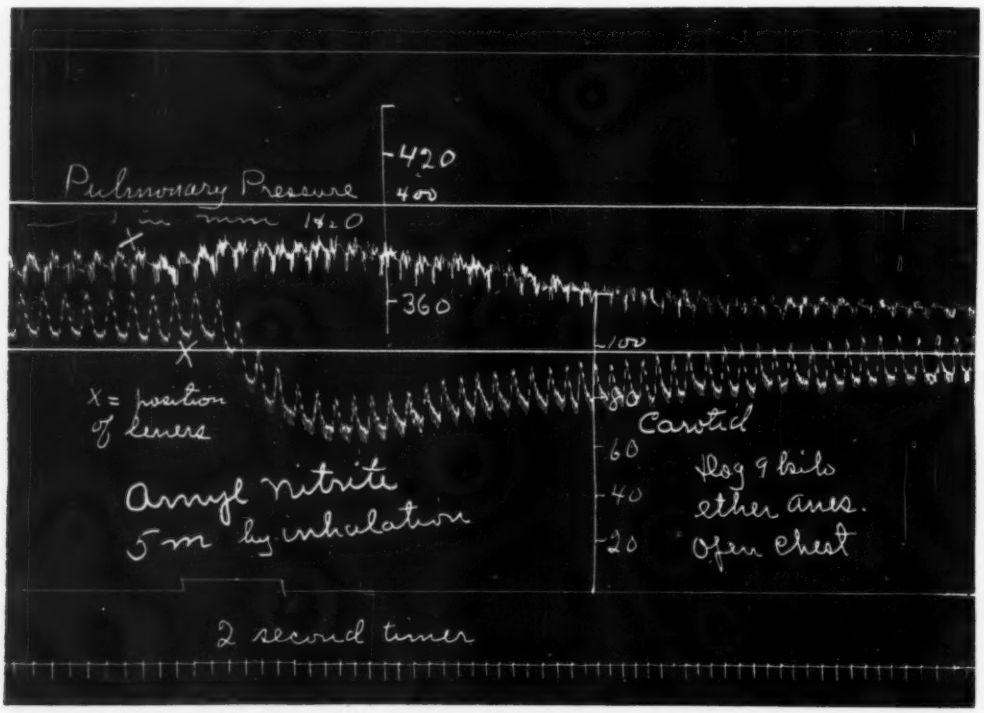

Fig. 1.—A common occurrence after amyl nitrite is a rise of pulmonary pressure, followed by a fall before the carotid pressure returns to normal.

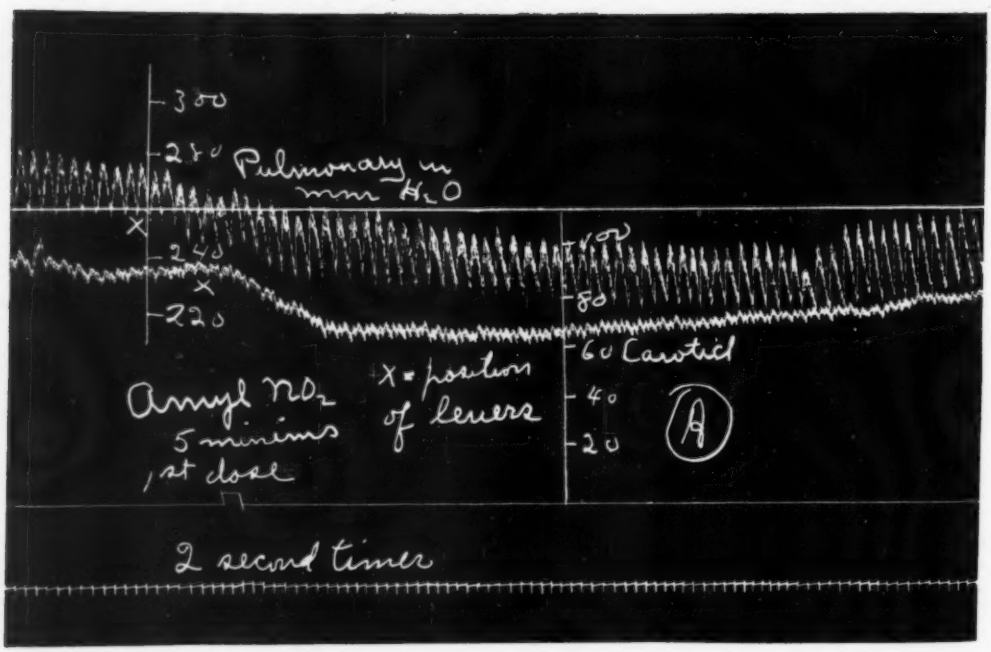

Fig. 2.—First dose of amyl nitrite produced a fall of both pulmonary and carotid pressures. (See Fig. 3.)

a gradual fall of carotid pressure following these drugs favors a fall of pulmonary pressure. (See Figs. 1, 2, and 3.)

Sodium Nitrite.—Sodium nitrite, 10 per cent solution, was injected intravenously in 50, 100, and 200 mg. doses. The smaller doses of sodium nitrite have an action upon pulmonary circulation similar to amyl nitrite and nitroglycerin; however, sodium nitrite produces a rise of pulmonary pressure less frequently than the other nitrites. The frequent failure of sodium nitrite in elevating pulmonary pressure (although amyl nitrite or nitroglycerin may raise the pressure in the same animal) is probably due to the more gradual fall of carotid pressure. (See Fig. 3.) Thus, the cardiac acceleration is less marked, and the compensation for the increased venous return is adequate.

Large doses of sodium nitrite (200 mg.) in a dog under deep anesthesia or after repeated smaller doses of sodium nitrite produce a prolonged fall of both pulmonary and carotid pressures, which is partly due to cardiac depression. In an animal in excellent condition (light ether anesthesia, rapid

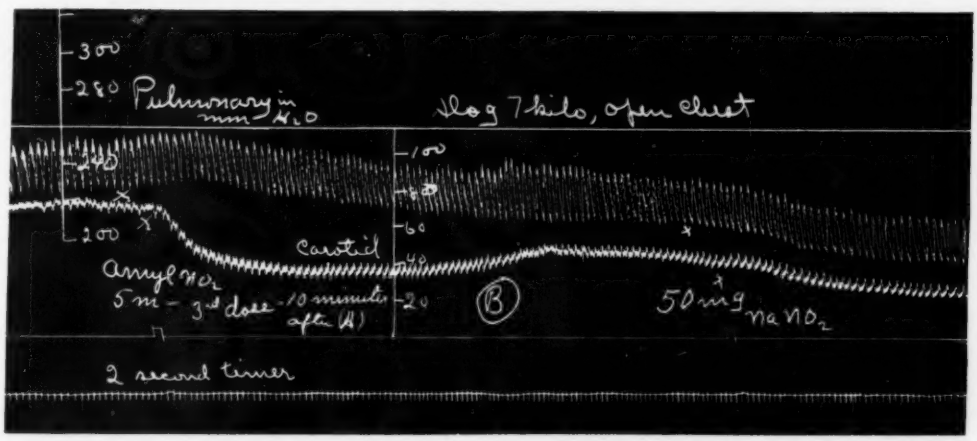

Fig. 3.—Third dose of amyl nitrite causes a rise of pulmonary pressure, followed by a slight fall. Note that carotid pressure falls more rapidly from this dose than in Fig. 2, due perhaps to some difference in preparation or administration.

technical procedures with minimal trauma and hemorrhage), a large single injection of sodium nitrite elicits a pressor action on both pulmonary and carotid pressures. We have made no effort to analyze this pressor action since it does not represent typical nitrite action. That the heart becomes more irritable during the pressor action is evidenced by the frequent appearance of acceleration, extra systoles, and fatal ventricular fibrillation. The action occurs from freshly prepared solutions and also from solutions one year old. We used two different brands of c.p. sodium nitrite (Bakers and Mercks), and dissolved the salts in distilled water.

EXCISED VESSELS

The excised pulmonary arteries of dogs were suspended in Locke's solution at 37° C. in a 30 c.c. container, with a constant stream of oxygen or air. In following the technic described by Macht,[5, 6] we observed no specific action of amyl nitrite or small doses of sodium nitrite on the pulmonary arteries,

but large doses of sodium nitrite (100-200 mg.) caused a powerful contraction of these vessels. This pressor action of sodium nitrite occurs after ergot and atropine. Also, after 1 mg. epinephrin, the addition of 100 mg. of sodium nitrite causes a further contraction of the pulmonary strip, equal to the original epinephrin stimulation. After the sodium nitrite contraction, the tissue, if washed and restretched, will again respond to epinephrin, barium chloride, or sodium nitrite.

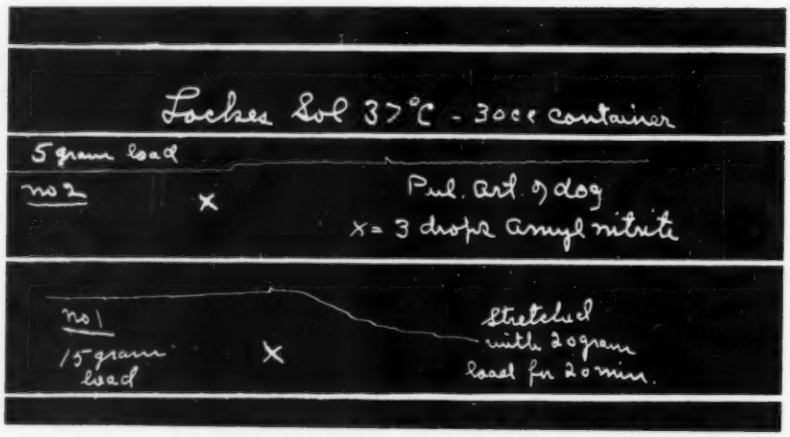
Locke Sol 37°C - 30cc container
5 gram load
no 2
Pul. Art. of dog
x = 3 drops Amyl nitrite
no 1
15-gram load
Stretched with 20 gram load for 20 min.

Fig. 4.—The excised vessel was treated as described in text. A 15 gram "relaxing load" was applied, and at No. 1 relaxation had ceased. Note the marked depression following amyl nitrite. The tissue was stretched with a 20 gram load for 20 minutes (without washing out), and at No. 2 the load was reduced to five grams. Note that now amyl nitrite has practically no effect as compared to sodium nitrite in Fig. 5.

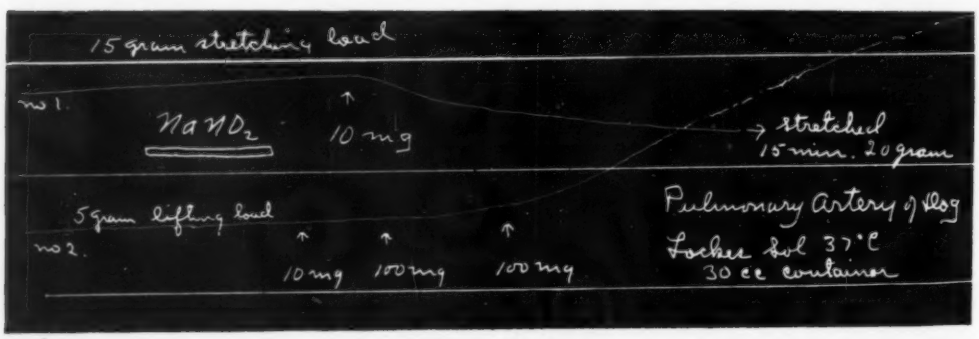

Fig. 5.—In No. 1 the pulmonary strip is relaxed by 10 mg. of sodium nitrite, using the technic described in text. In No. 2 Macht's technic was employed on the same preparation. Note that 10 mg. has no effect now, whereas the larger doses have a well-marked pressor action.

The method employed by Macht consists in stretching the pulmonary strip for fifteen to thirty minutes with a 20 to 30 gram load, then reducing the load to one-fifth or one-sixth of the stretching weights. This technic, as indicated in our previous paper,[7] is not adequate for testing smooth muscle depressants, since after thoroughly stretching the preparation, the extra-muscular tissue apparently will maintain the lifting load. We have modified the technic to demonstrate smooth muscle relaxation as follows: The fresh or ice box pulmonary strip or severed ring is adjusted in the

usual manner in a container (30 c.c. container, Locke's solution 37° C., oxygen or compressed air), and allowed to remain for a few moments without stretching. A load of 10 to 20 grams (depending upon size of the preparation) is applied on the lever while registering on a slow moving drum. After a short period of rapid relaxation, the recording lever usually strikes an horizontal line and sometimes begins to rise very slowly. If papaverine, amyl nitrite, or small doses (5-10 mg.) of sodium nitrite are applied at this time, relaxation occurs. (See Fig. 4.)

We have demonstrated that after a small dose of 10 mg. of sodium nitrite has relaxed the tissue, using the above technic, and the preparation is next weighted to 20 or 25 grams for fifteen to twenty minutes and the load is then reduced to from 5 to 10 grams, the repetition of 10 mg. doses of sodium nitrite produces no effect; but the addition of 100-200 mg. of sodium nitrite produces a marked contraction. (See Fig. 5.)

In perfused pulmonary vessels also, a small dose of sodium nitrite increases, whereas a large dose reduces the outflow. This is illustrated in the following experiment.

Perfusion Experiment No. 7.—Heart and lungs of 8 kilo dog removed under ether anesthesia. Intake cannula inserted through right ventricle into pulmonary artery and tied, outflow cannula inserted into left ventricle through aorta. Locke's solution 37° to 38° C. at intake cannula, pressure kept constant at 350 mm. H_2O. Injections made through T-tube intake cannula. Registration began as soon as heart ceased beating.

Outflow in c.c. per thirty seconds.

20
19
20
20

Injected 25 mg. $NaNO_2$ in 10 per cent solution, washed in with 5 c.c. Locke's solution 37° C.

23
22
19
19
19

Injected 200 mg. $NaNO_2$

27
12
10
6
9
14
18

Amyl nitrite causes an increase flow upon perfusion of the lungs, as may be seen in the following experiment. The amyl nitrite was introduced by breaking a 5 minim pearl in a rubber tubing off the intake T cannula and washing in the amyl nitrite with Locke's solution. (General technic same as preceding experiment.)

Outflow recorded in cubic centimeters per thirty seconds.

25
25
25
24

Injected amyl nitrite
42
33
26
23
23
22
23
24

Our work on excised dog pulmonary arteries may be summarized as follows: Nitroglycerin produces neither relaxation nor contraction with either technic. Amyl nitrite produces relaxation with the method herewith described, and no action with Macht's method. Sodium nitrite in small doses causes relaxation by our method, and no effect by Macht's technic. Large doses of sodium nitrite produce contraction by either method, but is more definite by the technic described by Macht.

DISCUSSION

The hypothesis, that the pressor action of nitrites upon pulmonary arteries is due to stimulation of the constrictor nerves, does not require our particular attention, since we failed to demonstrate a pressor action except in the case of very large doses of sodium nitrite. Since the pressor effect of large doses of sodium nitrite occurs equally well after ergot and atropine, we assume that the action is beyond the neuromuscular mechanism. The fact that large doses of sodium nitrite raise both carotid and pulmonary pressures and frequently cause ventricular fibrillation indicates that the pressor action upon the pulmonary circuit is not specific. The pressor action might be attributed to irritation and not true stimulation. Thus, Douglas[8] observed that a 5 per cent solution of sodium nitrite corroded exposed tissues so as to obscure the peripheral nitrite dilator action. Macht[9] found that sodium nitrite caused a contraction of the ureter, which he attributed to irritation and not to true stimulation. Cook[10] observed that sodium nitrite, when applied directly, increased the rate of the frog's heart.

CONCLUSIONS

Amyl and sodium nitrite, particularly amyl nitrite, often raise the pulmonary pressure in dogs, due to an augmented cardiac output from increased venous return and cardiac acceleration.

Large doses of sodium nitrite often produce a rise of both carotid and pulmonary pressures with cardiac irregularities, which sometime terminate in ventricular fibrillation.

On excised pulmonary arteries, amyl nitrite and small doses of sodium nitrite produce relaxation. Large doses of sodium nitrite cause a contraction of these vessels, which we suggest is caused by irritation and not true stimulation.

The action of amyl and sodium nitrite upon the pulmonary arteries is the same, though less marked, as upon the systemic vessels; however, the effect upon pulmonary pressure is often counterbalanced or overbalanced by the secondary effects of the action upon the systemic circulation.

REFERENCES

[1]Bradford, and Dean: Jour. Physiol., 1894, xvi, 37.
[2]Wood: Am. Jour. Physiol., 1901-1902, vi, 283.
[3]Wood: Jour. Exper. Med., 1911, xiv, 326.
[4]Petitjean: Jour. de Physiol. et de path. gén., 1908, x, 403.
[5]Macht: Jour. Pharmacol. and Exper. Therap., 1914, vi, 13.
[6]Macht: Jour. Am. Med. Assn., 1914, lxii, 524.
[7]Love, and McGuigan: JOUR. LAB. AND CLIN. MED., 1925, x, 882.
[8]Douglas: Jour. Am. Med. Assn., 1924, lxxxii, 381.
[9]Macht: Jour. Pharmacol. and Exper. Therap., 1917, ix, 427.
[10]Cook: Am. Jour. Physiol., 1909, xxiv, 263.

EDUCATION AS A CURE FOR PRESENT-DAY EVILS IN CLINICAL PATHOLOGY*

BY JOHN A. KOLMER, M.D., PHILADELPHIA, PA.

THE past fifteen years has been a period of especially rapid progress and development in clinical pathology, if we employ the term in its broadest sense to include those parts of clinical chemistry, clinical bacteriology and clinical serology constituting the work and scope of the average laboratory engaged in medical laboratory examinations. It is but natural that certain evils have crept into the field, as is always so likely to occur under such circumstances, and we hear much these days of the necessity for correcting them, this Society itself being dedicated to the task as one of its most important national activities. For several years I have been greatly interested in the situation and in a consideration of ways and means for improving the status of clinical pathology as a specialty in medicine, not only as a member of this Society, but likewise as Chairman of a Committee on Laboratories of the Pennsylvania State Medical Society, delegated to investigate conditions in this state and recommend remedial measures. At first I thought that the best way was probably the easiest way, namely, by legislation. But I now believe that legislation alone is probably insufficient, as it is comparatively easy to make laws but more difficult to enforce them, and I have finally reached the conclusion that education in a broad sense is probably one of the best and most reasonable weapons at our command, as has ever been true in the history of human progress. It will be my purpose this evening to define briefly some of the existing conditions which in a broad sense may be regarded as evils in the field of clinical pathology and to suggest how education may be reasonably expected to correct them in whole or in part.

As members of this Society we are dedicated to the principle that medical laboratory diagnosis or clinical pathology in a broad sense, should constitute a specialty in medicine just as surgery, ophthalmology and other clinical subjects are so regarded; but are we educating ourselves to fulfill this destiny? We recognize as an evil the taking over of this work by those who do not have

*President's Address delivered before the Fourth Annual Meeting of the American Society of Clinical Pathologists, May 20-23, 1925, Philadelphia, Pennsylvania.

a medical education, but does the merely technical phase of laboratory work demand such? Is it not true that many clinical pathologists are content to merely do the work which well-trained technicians are capable of conducting in just as acceptable a manner? If such is true we must first recognize the possible existence of an evil in our own ranks because clinical pathology will not readily win for itself the exalted position of a specialty in medicine as long as the subject is held to the merely technical phases of routine medical laboratory examinations. As so well and so frequently stated by our fellow members, Hillkowitz, Burdick, MacCarty and others, we must prepare ourselves to know disease in its broadest aspects and to serve our fellow practitioners as consultants in this field; we interpret the term to include, therefore, not only the training and experience necessary for the proper interpretation of routine examinations but the willingness to apply special measures for aid in the diagnosis of particularly difficult cases and to offer or even outline biologic and chemotherapeutic measures in treatment. In other words, merely to make routine laboratory examinations which a well-trained nonmedical technician may make just as well is not sufficient for elevating clinical pathology to a specialty in medicine, and the attitude of mind and practice that regards such to be the case is to be looked upon as an evil of first rate importance. If we insist upon a medical education as a requisite for the recognition of the clinical pathologist as a specialist in medicine, it must be under such circumstances as will draw upon medical education and experience; he or she must be willing and prepared by education to assume more responsibility in diagnosis than the technician is capable of assuming, and it is especially necessary to keep abreast of all advances in this field in order to be ever ready and willing for trying new methods of promise, possibly to conduct some original research, to keep in touch with clinical medicine and surgery and, lastly, as previously stated, to be prepared to advise in treatment and especially in the treatment of infectious diseases by biologic and chemotherapeutic agents.

Upon us falls the responsibility of proving that clinical pathology is a specialty in medicine deserving of wider recognition and adequate compensation. Just as the physician cannot hope to be successful by merely doing the work of a well-trained nurse, or the roentgenologist hope to establish himself as a specialist by merely doing the work of a technician in this field, so likewise the clinical pathologist cannot hope to qualify as a specialist by merely conducting routine laboratory tests readily handled by nonmedical technicians. He must be prepared by education and experience to do more, to shoulder more responsibility when occasions arise and by these means prove that clinical pathology in its broad sense is indeed a real and important specialty in modern medicine, full of opportunities and promising in financial returns, rather than a good camping ground for the misfits and all and sundry who choose to enter the field.

That we have a real need for the laboratory technician cannot be denied. Indeed, I believe the well-trained technician is almost as indispensable in the laboratory as the nurse at the bedside. I am sure that a hospital or a community without a good pathologist but with a good laboratory technician is

much better off than a community or hospital without either. But unfortunately the true functions of the technician are not as widely and clearly understood as they should be and the tendency for them to actually practice medicine has amounted in some localities to an evil requiring adequate measures for correction. There is today a wide field of usefulness for well-trained technicians, not only as assistants to practitioners of medicine too busy to conduct their own laboratory examinations, but likewise as assistants to pathologists whose time can or should be far more profitably spent than doing the simple routine laboratory tests. Naturally a large number of practitioners wish merely to hire someone to conduct the tests, while leaving the matter of interpretation of results for themselves. And as long as this is actually the case I can see no harm in the situation, but not infrequently the technician assumes, or has forced upon him or her, the latter function which may automatically make them diagnosticians and thereby in a certain sense practitioners of medicine, since their information not infrequently reaches the laity direct. How to correct this situation is one of the most perplexing questions of the present day. The remedial measures must not deny the physician or institution the right to employ the technician. Legislation may readily define the limits of their activity and forbid the nonmedical individual from infringement upon the practice of medicine but it is one thing to spread laws on our books and another to enforce them; furthermore, our state boards of medical licensure have their hands full in dealing with far more dangerous and pernicious infringers of the various medical practice acts than the laboratory technicians. In the correction of this evil I believe that education is particularly full of promise, and I refer especially to the education of the practitioner of medicine, secondarily to that of the laity and lastly to that of the technician.

I believe it may be stated without reserve that if the medical profession so will it, every irregular and incompetent laboratory and technician could be put out of business by a lack of patronage. It is true that some commercial laboratories flourish by the patronage of the laity but in a general way they confine their activities to the examination of the urine and it is to be hoped that in time the laity could be educated to an appreciation of their very limited value and quackish character.

But there is a real need for the education of the medical profession in two main directions, namely, the education of those physicians who employ the laboratory to exercise more care and discretion in the choice of the laboratory to which they entrust their work and, secondly, the encouragement by education of those physicians who do not use the laboratory at all or but seldom, to a better realization of the aids in diagnosis and treatment offered by the well-trained pathologist and adequately equipped laboratory. If physicians could be more broadly educated to look upon clinical pathology as a specialty in itself through the efforts of clinical pathologists to make it so, and to refer their work to medically trained pathologists, the field would be more rapidly developed, more rapidly placed on a satisfactory basis for clinician, pathologist, and layman alike and render the specialty of clinical pathology more attractive to the medical man or woman as a field for practice.

Indeed I doubt if anything more need be done to correct the existing evils, and this Society, in cooperation with other national societies, may do well to launch such an educational campaign through the state and county medical societies, while establishing at the same time standard qualifications for both pathologist and laboratory and aiding institutions and communities in securing both.

It is certain that at the present time there is not a sufficient number of qualified pathologists to supply the needs of the country at large; it is also certain that the supply is insufficient because the specialty has not been made sufficiently attractive for graduates in medicine and, finally, it is certain that unqualified individuals have stepped into the breach to supply the demand. A pernicious cycle must be broken and in my opinion it can be best broken by a well-directed educational campaign. I am convinced that many more of our young graduates in medicine would take up clinical pathology as a specialty if it were made more attractive; I myself cannot encourage any young medical man to enter this field merely as a competitor of the laboratory technician with the comparatively low professional and financial returns, but the situation could be changed and the field rendered increasingly attractive by better cooperation and the exercise of better judgment on the part of practitioners of medicine and surgery and these results are to be obtained only by education of the latter. One is tempted to state that this education should begin in our undergraduate medical schools but our educators and students alike are so harassed by the multitude of subjects to be taught and learned that one hesitates to add to their burdens, although it is to be stated that many of our medical schools could greatly improve their teaching in this field by providing better facilities for the teaching of clinical pathology during the third and fourth years; but of far more importance than the teaching of mere technic is the matter of the teaching of the broad and very practical applications of clinical pathology in the diagnosis and treatment of disease and the necessity for having the work done well if it is to be done at all. In a general way, however, we see the results of such teaching at present among our younger practitioners; it is among the older ones, those who did not have such opportunities for instruction in their undergraduate days, that we see most need for education in these matters. This education should consist in the proper evaluation of laboratory examinations as a safeguard against depending upon them either too much or too little and to show physicians that the work is best done and that they will be best helped by calling upon the medically trained clinical pathologist to at least supervise such examinations and to serve as a consultant should the case require expert knowledge in this particular field.

I may pass over even more hurriedly the subject of education of the laity except to state that education of the medical profession should be first, since there is no better educator of the layman than his medical adviser. But in addition, a great deal may be done by a well considered educational campaign through such publications as *Hygeia,* the magazine of health published by the American Medical Association, by radio talks and even newspaper articles prepared and disseminated by such responsible medical associations as our

county and city medical societies. Indeed, I am inclined to believe that the laity is following us so closely in all that pertains to the eradication of the unworthy in medicine, that they may furnish some of the stimulus and incentive to the medical profession for cleaning house in matters pertaining to the diagnostic laboratory.

At any rate I believe the time is ripe for a wider recognition of existing evils and an earnest consideration of education along these various lines for their eradication. I have the impression that we are only waiting for leaders to appear with definite ideas and definite plans, and I believe that the American Society of Clinical Pathologists is the logical body to undertake the task, working in heartiest cooperation with any other national or local society imbued with the same ideals and purposes. Education is slower than legislation but it is the surer and better way; this Society has already made a good start and the task may not be as great when actually under way as we view it now from a distance.

At the present time our attention is commanded by discussions and experiments in the standardization of laboratories since so much has been accomplished by the American College of Surgeons in the standardization of certain hospital activities. But I am sure that the matter of standardization of laboratories arouses in others the same conflict of opinions and emotions as it does in me. It is right, proper and helpful for the State to insist upon the laboratories of institutions receiving state aid to possess a minimum of equipment and an adequate personnel since the public funds are being expended; likewise in the case of those laboratories to be recognized as affiliated with the state laboratory for examinations in the infectious diseases after the plan developed by Wadsworth and Gilbert in New York. But the State has no more right to inspect or meddle with the work of the private laboratory of the duly examined and licensed medical graduate practicing clinical pathology than it has with the office and practice of any other physician, and due care in this direction must be exercised against socializing medicine. I am heartily in favor of empowering our State Boards of Medical Licensure to exercise a more adequate control over laboratories being conducted by non-medical individuals because the health of individuals and even of the public may be and is now being jeopardized by incompetents in this field, but I believe that most will be accomplished in time by our Society establishing minimum qualifications for the specialist in clinical pathology, providing for an examining board and having her certificate recognized by an educational campaign as the badge of approval on the clinical pathologist bestowed by those in best position to judge of the qualifications and equipment of the applicant, for the guidance of institutions, the medical profession and even the laity seeking the services of a real specialist in this field of practice.

And while we are considering the State in relation to the correction of existing evils in laboratory medicine, may I suggest that the present activities of many state and municipal laboratories are so rapidly socializing this field of practice as to constitute a growing evil? No one will deny that these laboratories have done and are doing a great service in relation to the diagnosis and control of the infectious diseases and there is a real need for them in this

field of work since the public is involved, but the present tendency for doing all kinds of laboratory work free of charge and making the most strenuous efforts to obtain it for presenting statistical evidence of the growing popularity and activities of the laboratory or its director to all who may be subject to impression by such should be curbed as a growing evil, unless we entertain the opinion that medicine should be socialized and that here is the best possible start toward this end.

Before concluding these remarks I wish briefly to refer to two other educational matters in relation to clinical pathology, namely, the education of the technician and of the hospital interne, since these are fields in which we may all contribute a share. As previously stated I believe the well-trained laboratory technician is a blessing and indispensable for the proper conduction of the medical laboratory. But let us better realize our responsibility in his or her training, aiming to teach them good and acceptable methods, the necessity for hard and conscientious work and that there is no place for the careless worker interested alone in the financial returns or for the "sink-test specialist." In the final analysis the majority learn by observing the way we do things, and it is our duty to clearly define for them what they may readily learn to do well and acceptably and that, beyond the purely technical phases, a broad and comprehensive medical education becomes increasingly valuable. I am convinced that laboratory technicians are yielding on the whole valuable service to the medical profession and there is such an increasing demand for their services, and especially as assistants in institutional laboratories, that the situation may well command the attention of this Society as an additional field for service.

For example, I should like to see our Society look into the matter of present-day methods for educating technicians and to establish minimum standards; also to establish a bureau of examination or at least of registration to which physicians and institutions may look for aid in obtaining the services of a qualified technician. In this connection I may state that the training of technicians has commanded my attention for the past eleven years and the system now in vogue in the laboratories of the Graduate School of Medicine of the University of Pennsylvania is proving very successful and may be readily adopted by any large laboratory. In the former Polyclinic and College for Graduates in Medicine I gave a formal course of instruction in clinical pathology, clinical bacteriology and clinical serology embracing lectures and practical laboratory work. But when this institution became amalgamated a few years ago with the University of Pennsylvania, the dean of the Graduate School of Medicine, Dr. George H. Meeker, decided that these courses should be dropped in order to permit us to better take care of the increasing numbers of student graduate physicians. Realizing that the training of technicians was rendering a distinct service I then proposed, and the Dean promptly accepted and endorsed, the present system of accepting a few students each year under a volunteer assistantship plan by which the applicant agrees to serve in the laboratories on a full time basis for a period of sixteen months. This time is divided into four periods of four months each in the laboratories of histologic technic, clinical pathology and bacteriology, blood chemistry

and serology. Each laboratory is in charge of a highly trained assistant under whom the volunteer works and learns by actually assisting in the work of the institution from the very first to the very last day of the course. The work is entirely practical and technical, supplemented by evening reading and study. The University now requires each appointee to make a deposit of $160 but this is returned at the rate of $10 per month, making the course entirely free of tuition. The plan has worked admirably and the technicians are certainly acquiring a much better training and have a much better understanding of their real capacities and functions than was possible under the former plan. It is true that they require instruction and that the plan is bound to fail unless the instructors are in sympathy with the idea, but the volunteers soon learn to become useful and the plan is truly and indeed upon a fair and just *quid pro quo* basis. I am hoping very much that other laboratories will take up the plan which is after all nothing more than a refinement of the oldest plan of all, namely, the apprentice system, and I am in position to state that the laboratory is able to conduct a much larger volume of work without an increase of the salary budget than is otherwise possible, while a distinct and valuable service is being rendered by furnishing a source of supply of fairly well trained experienced laboratory technicians who are just technicians without fake notions of being pathologists or physicians.

In conclusion I wish to enter a plea for more care in the education of the hospital interne entrusted to us for training and experience in the laboratory. Here is a golden opportunity for education along the lines so necessary in my opinion for the advancement of the cause of clinical pathology. Too frequently the interne is allowed to do much as he pleases or is required to spend his valuable time in the simplest kind of laboratory work like urinalyses and blood examinations. No wonder that under such conditions the majority of internes look upon the laboratory service as something to be endured rather than an opportunity to be welcomed. It is true that their function is to serve the institution but it is equally true that they have every reason and right to expect instruction and the opportunity to learn and I would suggest that we embrace this opportunity for educating these young men and women into a proper appreciation of laboratory medicine by giving them more of our time and instruction, more opportunities for assisting in a variety of laboratory examinations of all kinds instead of tying them down to a fixed and monotonous routine which turns them out with the idea that clinical pathology is merely a field for the nonmedical technician, instead of warranting their serious consideration as a specialty in medicine well deserving of their closest attention and study.

THE RÔLE OF THE CLINICAL PATHOLOGIST IN HOSPITAL EFFICIENCY*

By Malcolm T. MacEachern,† M.D., C.M., Chicago

I HAVE selected this subject for my remarks because I am fully convinced that the clinical pathologist plays an important rôle in hospital efficiency. It, therefore, affords me much pleasure to discuss this subject with you for a few minutes.

The American College of Surgeons ten years ago formulated a minimum standard of requirements for hospitals. This standard consists of broad, adaptable and adjustable principles. One of these principles is of interest to the American Society of Clinical Pathologists inasmuch as it concerns clinical laboratory service in hospitals. I refer to Clause 5 of the Minimum Standard, which reads as follows:

> "That diagnostic and therapeutic facilities under competent supervision be available for the study, diagnosis and treatment of patients, these to include, at least (*a*) a clinical laboratory providing chemical, bacteriological, serological and pathological services; (*b*) an x-ray department providing radiographic and fluoroscopic services."

The above requirement presupposes the following considerations:

I. ADEQUATE ACCOMMODATION

Proper spacing, planning, airing, lighting and accessibility are desirable for the clinical laboratory. Fortunately, in recent years, there is a tendency to depart from the custom of placing this department in the basement. It is generally agreed today that the clinical laboratory deserves as desirable a location as any other department in the hospital. Personally, I have been advocating its location in or adjacent to the operating room suite. The best location, however, can be selected by determining where contact will be afforded with the greatest number of the medical profession at all times.

There has been too much isolation of this department in hospitals in the past. The old saying, "Out of sight, out of mind," applies in this case. Isolation tends to minimize use and service to the profession. No doubt that with the rapidly growing importance and increased use of the clinical laboratory, more attention will be directed towards the better locating of this department.

It is also desirable, where possible, to make the necessary physical divisions as to table and wall space for the various activities carried on, namely, chemical examinations, clinical microscopy, bacteriology, serology, pathology and blood chemistry. This tends to more efficient operation.

*Address delivered before the Fourth Annual Convention of the American Society of Clinical Pathologists, May 22, 1925, Philadelphia, Pa.

†Associate Director American College of Surgeons and Director of Hospital Activities.

A number of the larger hospitals today find it advantageous to have one or more smaller working laboratories scattered throughout the wards and subsidiary to the main department. Frequently we find the tissue laboratory placed in or adjacent to the operating room suite, so as to be more readily accessible for gross and microscopic examination of all tissues removed at operation. An arrangement of this kind has three distinct advantages: (*a*) It minimizes errors in identification of specimens. (*b*) It tends to better preservation of natural appearances and qualities of the specimens removed by shortening the time of transportation. (*c*) It affords closer association between the surgeon and the pathologist. This arrangement is always commendable.

Ward laboratories are of distinct advantage to the clinician and the interne, providing they are under proper supervision. Personally, I favor the consolidated laboratory system where the facilities, technic and personnel can be kept under constant proper supervision. I would, however, make one exception to this, as mentioned above, that is, the having of the tissue laboratory in or adjacent to the operating room suite if the whole department cannot be so located. I believe that too many small laboratories scattered throughout the institution renders proper supervision of equipment, technic and workers impossible, and, therefore, inaccuracies in technic and interpretation may result. We all realize full well that bad laboratory work is worse than none at all.

II ESSENTIAL FACILITIES

It is generally agreed upon that there are five branches of work carried on as a minimum in the average laboratory today. These are: (*a*) Chemistry or Clinical Microscopy, (*b*) Bacteriology, (*c*) Serology, (*d*) Pathology, (*e*) Blood Chemistry. Every hospital requires these services in order to do proper work and discharge its full responsibility in the best scientific care of the patient. Laboratory equipment is today fairly well standardized and, therefore, I need not dwell on this phase of the subject.

In passing, may I suggest that I believe we must look to this society for standards in planning, equipment, personnel and technical procedures? There is a continuous demand from the field in this respect, and authorized information from such a body as yours would be greatly appreciated and of value to the hospital field. It is necessary today that the hospitals be provided with carefully worked out minimum standards for the various activities carried on. Given these standards they will build up the service more efficiently in approximating a maximum.

III. EFFICIENT PERSONNEL

A fine clinical laboratory with excellent appointments and facilities does not always mean efficiency. This, of course, is desirable, but it is the personnel of the department which counts most. This personnel generally falls into three divisions:

1. *Supervising or Directing.*—The supervising and directing of the work of the clinical laboratory can only be done efficiently through a well-trained,

competent clinical pathologist, part or full time, depending on the nature and amount of work to be carried on. It is unfair to throw responsibility of laboratory work on the technician, both from the standpoint of complicated technic and interpretations.

While most hospitals today have satisfactory technical services the American College of Surgeons believes and requires that the clinical laboratory be under the supervision and direction of a competent clinical pathologist. A fundamental medical knowledge with subsequent special training and experience is essential for proper and accurate interpretation of findings, as well as for the development of technic.

We are informed on good authority that there are between seven and eight hundred clinical pathologists available in the United States and Canada, to take care of the work in some seven or eight thousand hospitals, of which approximately twenty-five hundred are active general hospitals of thirty-five beds and over. It is, therefore, quite evident that there is distinct shortage of well-trained clinical pathologists. The best solution for this problem at present is the grouping of hospitals under one clinical pathologist, providing sufficient technical service in each institution to do the work efficiently.

2. *Technical Service.*—Technicians render most excellent service under competent supervision. This work, I find, is usually taken up by nurses and university graduates. The nurse technician in the smaller hospital, where her full time is not required in the laboratory, is able to assist with other work. Not infrequently do we find many combinations of services effected, as for instance: (*a*) Laboratory and various types of nursing or administrative activities in the hospital; (*b*) Laboratory and x-ray technic; (*c*) Laboratory and case records; (*d*) Laboratory and x-ray technic and case records. However, in all these combinations let us avoid placing too many duties on the technician. In most instances a technician can be kept busy in any one of the above mentioned services.

I cannot dismiss this part of my discussion without emphasizing the importance of having properly trained technicians. Many instances are known where laboratory errors have been most embarrassing, not to say anything of the damage they may have done to the patient. Let us not forget that untrained and improperly supervised technicians are very dangerous in any institution.

3. *Janitor Service.*—Every laboratory requires competent janitor service to keep the department clean and orderly. Not infrequently we find this feature overlooked, to the detriment of the worker and the efficiency of the department. A good janitor service will embrace: (*a*) Keeping the room and equipment clean and orderly. (*b*) The proper storage and care of bodies. (*c*) The feeding and care of the animals used for laboratory purposes. (*d*) The assisting with certain procedures as required. Many other duties are generally attached to this service.

IV. PROPER ORGANIZATION

The clinical laboratory is one of the important service departments or units in the entire organization of the hospital. It is one of the contributing

units in service to the patient and comes under the medical division, taking its place both in the diagnostic and therapeutic aspects of medicine. It must be thoroughly organized. As already stated, a department head or director is necessary, who shall be responsible to the hospital management for the operation of this particular department. Assistants, technical staff and others should be directly responsible to the head of the department.

The entire staff must be imbued with earnestness in their work and the doing of everything to render the best service possible to the patient. They must also be imbued with the spirit of cooperation, coordination, efficiency and economy in the management and operation of the department. Underlying the technical service there must be a distinct personal interest in the department itself as a unit of the larger or more complete organization of the hospital.

V. LABORATORY RECORDS

A good record system in the hospital today consists of many units contributed by the various departments concerned. One of these units is that of the clinical laboratory, which should establish and carry out a definite, simple but thorough record system. The following outline may be considered as a basis for such a system:

1. *Requisition for Work Desired.*—This form should be applicable for all examinations. Ample space to give a very brief abstract of clinical data is desirable. Too many requisitions come to the laboratory with indefinite requests and information, thus placing the clinical pathologist at a distinct disadvantage. A comprehensive requisition method will not only expedite matters but will assist the clinical pathologist in giving a more intelligent service.

2. *Report of Findings.*—This requires to be made in duplicate, one copy to go to the patient's file and the other to remain in the department as part of the record system.

3. *Daily Report.*—The daily report shows the various consecutive examinations made each day. This may be recorded in a loose leaf system.

4. *Filing of Reports.*—Usually the vertical folder and cabinet is used for the filing of the report, arranged alphabetically or according to the patient's number.

5. *Cross Index.*—Many methods of cross indexing laboratory reports are found. A good system makes it possible to find data by name, hospital number, and at the same time provide the necessary grouping of the various types of examinations and findings.

Frequently it is found preferable to assign a laboratory number to each requisition. If this is done it must not be so placed as to confuse it with the hospital number, which should be carried throughout. Requisitions may be filed under the doctor's name. In this way the amount of laboratory work called for by each doctor is readily checked up.

A summary laboratory sheet has been found desirable and practical in many hospitals. This sheet immediately affords the doctor in charge of the patient a bird's-eye view of the amount of laboratory work done over any given period and saves him from searching through many forms.

VI. LABORATORY CHARGES

A great deal has been said and written during recent years in regard to laboratory charges. However, it still remains a matter of much diversified opinion as to the best methods for a hospital to adopt. A review of the situation indicated the following methods in use at present:

1. Adoption of a schedule of charges for the various tests performed, varying according to the nature of the examination.

2. Including of this service in the *per diem* rate charged to the patient.

3. Adoption of a flat rate to include all the laboratory work.

4. Providing a flat rate for some of the routine work, and making a charge for all other examinations required.

5. Providing a free service, as might be granted through the federal, state, county or endowed laboratory.

6. Turning of the work over to a clinical pathologist who secures part or all of the fees and provides the necessary free service.

The American College of Surgeons has not recommended any particular system, but urges that whatever method is used it should not limit or embarrass the amount of work called for and required in the best interests of the patient. It is hoped, however, that the near future will see a more uniform policy of charges worked out for hospitals.

VII. ROUTINE EXAMINATIONS

There is also much diversified opinion as to what examination should be routinely carried out in the hospital today. The following are generally given the most consideration in this respect: (*a*) Urinalysis; (*b*) Blood examinations, red, white and hemoglobin; (*c*) Blood coagulation; (*d*) Wassermanns; (*e*) Tissues from operation. The American College of Surgeons has left this matter to each hospital to work out, with the exception that they insist on the routine urinalysis and tissue examinations. Considerable variance of opinion exists as to whether tonsils should be examined pathologically or not. However, I am pleased to state that each year more and more hospitals are including the pathologic examination of tonsil material in their routine work.

VIII. CLOSE CONTACT BETWEEN THE CLINICAL PATHOLOGIST AND THE MEDICAL STAFF

It is desirable that the closest contact be maintained between the clinical pathologist and the medical staff. In the past this has not always been the case. The clinician and the clinical pathologist must work hand in hand if we are going to get the best results. This can be brought about in several ways:

1. Placing of the laboratory in a location accessible to the members of the medical staff, where contact is much more frequent. The laboratory should be open to the clinician at any time he wishes, and he should always receive as much personal attention as can be afforded him.

2. Placing of the tissue laboratory in or adjacent to the operating room suite will bring the surgeon and the clinical pathologist into much closer contact. The clinical pathologist should be available for the surgeon when he is operating, if he requires him. It may be an advantage to the surgeon to have

the clinical pathologist see the tumor before it is removed and advise with him in regard to its removal. It is advantageous to the surgeon to have the clinical pathologist demonstrate the freshly removed tumor to him immediately after operation. In this way the surgeon keeps abreast with his gross pathology. In addition, the mutual study of the gross specimen in a fresh state will usually prevent difference of opinion arising between the surgeon and pathologist later as to diagnosis. Again, the surgeon may follow the section through the laboratory and study the microscopic findings. There is no doubt in the world but what the surgeon becomes a better surgeon from contact with the clinical pathologist, and the clinical pathologist becomes a better pathologist from his contact with the surgeon. Therefore, let us stimulate this teamwork in hospitals as much as we can.

3. The clinicopathologic conferences now held in all well-regulated hospitals are of untold value in bringing the clinical pathologist and the medical staff closer together. They have made staff conferences more interesting. The findings of pathologic conditions, ante- and postmortem, in themselves cannot be of much worth unless made use of and studied in relation to the living and normal. The clinicopathologic conference should embrace gross and microscopic pathology. The subject matter of such a conference may be presented under four headings:

(*a*) History of the case. (*b*) Demonstration of gross and microscopic findings, either through the actual specimen or lantern slides. (*c*) General discussion of findings in relation to history. (*d*) Conclusions to be drawn from the study of this material.

A careful record of the clinicopathologic conference should be kept and the discussion and findings attached to the respective case histories discussed.

The clinical pathologist must not be regarded in future as a mere technician to work in his laboratory and not mingle with the other members of the profession. More and more he is being called in by the clinician as a consultant at the bedside of the patient. Not infrequently the clinical pathologist may render the clinician material assistance in determining new lines of procedure as to diagnosis and treatment. And this is as it should be, for the fundamental principle of hospital standardization as advocated by the American College of Surgeons is the surrounding of the doctor or clinician, be he surgeon, physician or specialist, with all that is available in medical science in order that he may give his patient the maximum service in diagnosis, treatment and end-results obtained. Unfortunately, in a few instances, there is a feeling of lack of confidence in the clinical pathologist by the surgeon, who is sometimes skeptical as to the value of the pathologic service to the hospital. This can be remedied only through a working contact as I have endeavored to outline.

IX. PERIODIC CHECK-UP ON WORK AS TO QUANTITY AND QUALITY

The clinical pathologist must constantly check up on the laboratory service as to quantity and quality. A hospital may be doing too much or too little laboratory work for the patient. This can be determined from a study of each case, and especially should be noted through the staff conference

analysis. The clinical pathologist must always keep a watchful eye over the quality of the work. In checking this up he should have access to the patient in order to compare clinical findings with laboratory findings. In examining a hospital some time ago I found they had a wonderful laboratory, excellent equipment and splendid personnel. Out of the entire volume of work done during the year there were only six tissues examined, notwithstanding the fact that there were fourteen hundred and twenty-six operations, with approximately seven hundred showing removal of tissue in one form or the other. While this hospital was doing very creditable laboratory work, it seems to me they failed to carry out one of the essential services. The American College of Surgeons has found it necessary to check up the quantity and quality of laboratory service in all hospitals surveyed. It is difficult to determine the quality of laboratory service, but comparatively easy to ascertain the quantity. The monthly analysis sheet, which we require each hospital to fill in, contains on the reverse side a detailed report of the various laboratory examinations made each month.

X. DIFFICULTIES ENCOUNTERED IN THE SMALLER HOSPITALS

We have met with difficulty in establishing efficient laboratory service in the smaller hospitals, particularly in getting this work under competent supervision. It is not difficult to maintain a good technical service in these institutions. Frequently part of the service can be secured from a neighboring institution or through the federal, state or municipal laboratory. However, we insist that every hospital makes provision for the emergency examinations that are necessary. By this I mean such examinations as are essential in assisting the doctor to make his diagnosis as quickly as possible. The American College of Surgeons believes that every hospital should be able to do the following tests without delay: urinalysis, blood examinations, coagulation, smears and spinal fluid. Serology, pathology and some of the other tests can be sent out for report. The American College of Surgeons, in its hospital standardization program, has adopted the rule that necessary laboratory service should be available without delay, regardless of where it is to be secured from.

Supervision of the smaller laboratory has been difficult. In many instances a clinical pathologist is not available for either part time or full time duty. Occasionally one of the medical staff now in clinical work, but who has had previous pathologic training and experience, is secured to assist in supervising the department until better arrangements can be made. The grouping of hospitals under one clinical pathologist, with technical service in each, has helped considerably to solve this problem.

XI. CONCLUSION

I cannot conclude this paper without making reference to the great increase in hospital efficiency which has resulted through the establishment and development of the clinical laboratory during the past few years. I believe that the clinical laboratory has done much to improve medical service, especially in the following respects:

1. The assisting of the clinician in making a more accurate and intelligent diagnosis. The clinician today uses the clinical laboratory in numerous ways

to assist him in making or confirming diagnoses. The many available examinations have made diagnoses more accurate, as well as shortening the time in working them out.

2. The eliminating of unnecessary, incompetent and illegal surgery. No longer do we find the specimen from operation thrown away in the bucket. Now, in properly regulated hospitals, it is handed over to a competent, well-trained clinical pathologist who analyzes it carefully and describes it macroscopically and microscopically. These recorded findings are thoroughly reviewed by the staff of the hospital and thus incompetent, unnecessary and illegal surgery more readily ascertained.

3. The stimulating of a more thorough clinical interest on the part of the staff through the clinicopathologic conference. The study and demonstration of gross and microscopic tissues in relation to the clinical findings tend to vitalize staff conferences and increase much greater interest.

All the above has contributed much towards improving medical service in hospitals. The American Society of Clinical Pathologists has done a great deal towards promoting the cause of better laboratory service in hospitals. Its assistance is still very much needed. Being deeply interested in hospitals and the improvement of service, I am almost bold enough to lay out a program for your society which I am sure would do much to elevate our hospitals to a higher plane of efficiency. Personally, I feel that there are five problems to which this society should give attention. Perhaps it has done so already. Here are my suggestions:

1. The stimulating of the training of more clinical pathologists.

2. The standardizing of the training of clinical pathologists and technicians.

3. The standardizing of planning equipment and facilities for laboratory work.

4. The making of a survey of the United States and Canada for the purpose of determining the number of available clinical pathologists and their credentials.

5. The preparing of a list of text and reference books and magazines suitable for medical libraries in hospitals.

In conclusion, let me thank you for your support in our hospital standardization movement which today embraces over twenty-four hundred hospitals in the United States, Canada and other countries. Again let me call your attention to the fact that this movement is based entirely on service to the patient, and taking into consideration all the contributing units, the clinical laboratory with its service is one of the most important.

A STUDY OF TISSUE AUTOLYSIS IN VIVO*

II. A Pharmacological Study of the Toxic Material

By Edward C. Mason, M.D., and Edward C. Davidson, M.D.
Assisted by P. B. Rastello

IN a previous paper on this subject[1] we have called attention to the fact that the autolysis of a small amount of liver tissue free within the abdomen is accompanied by marked and constant changes in the animal's blood chemistry. We also expressed the opinion that such chemical changes did not give an adequate explanation as to the cause of death accompanying the autolysis.

The present study was undertaken with the hope of gaining more definite information as to the (1) nature of the toxic material, (2) its degree of toxicity and (3) its mode of action. During the study, observations were made using (1) extract of fresh ground liver, (2) extract of autolyzed liver, (3) abdominal fluid present at the time of death accompanying autolysis, and (4) nonprotein fraction of the autolyzed liver extract. Since completing the experimental portion of this work it has been our pleasure to read the splendid report of Cannon[2] on the "Evidence of a toxic factor in wound shock." Although we have approached the problem from an entirely different angle there appear to be many points in our observation which are subject to interpretation in terms of Cannon's report. He has made the distinction between *primary* and *secondary* shock, and it is especially to the latter which we wish to refer. In the study of "wound shock" Cannon describes his experiment as follows: "In order to bring about, in lower animals, a traumatization similar to that giving rise to shock in man, the thigh muscles in the anesthetized cat, while being supported by an iron block, were repeatedly struck with a blunt wedge-shaped hammer, or they were crushed by compression. The trauma usually failed to break the skin, so that infection from without was impossible." After about twenty minutes the blood pressure of such animals began to fall, and after about one hour it had usually fallen to a shock level. The possible effect of anesthesia, hemorrhage, reflex inhibition of the vasoconstrictor center, exhaustion of the central nervous system with loss of vasomotor tone, acapnia and fat emboli were ruled out and the circulatory mechanism was then studied as the most reasonable point of attack of the liberated toxic material.

That the toxic material is carried in the circulation was demonstrated by Cannon in the following manner: the blood vessels of the leg (the iliac artery and vein) were ligated before the muscles were crushed, and the ligatures left in place for thirty-three minutes after the trauma, during which there was no fall in blood pressure. However, as soon as the blood flow was reestablished, the pressure promptly fell to a low level.

*From Henry Ford Hospital, Detroit, Michigan.
Received for publication, October 16, 1924.

EXTRACT OF FRESH NORMAL LIVER

Extract of fresh normal liver was prepared by grinding the tissue with washed sand and extracting the mass with 0.9 per cent sodium chloride solution. The amount of saline added was equal to the original weight of the tissue used. After extracting for ten to fifteen minutes, the whole was filtered through cheesecloth. Fig. 1 is presented to show the effect of intravascular injection of such tissue extract, the record being obtained from a dog weighing 10.4 kilos and represents respiration and blood pressure. The animal received five injections of tissue extract, the dose increasing from 1 c.c. to 16 c.c. With each injection there is shown a definite blood pressure response. Yet promptly after each fall in blood pressure the pressure returned toward normal, there being apparently no prolonged toxic effect. This toxic action of normal tissue extract

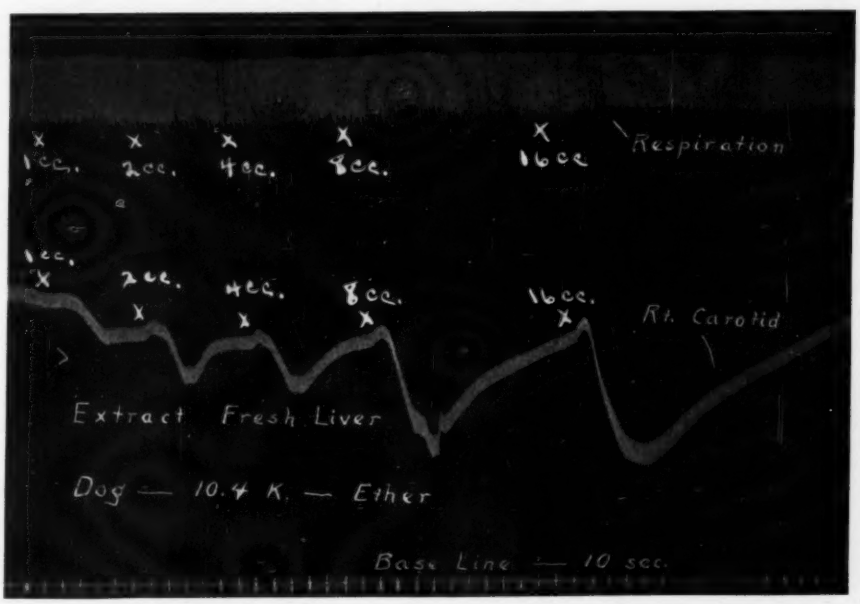

Fig. 1.

has been studied by various observers and has been ascribed to principally one of two causes or to a combination of the two; (1) the intravascular coagulation resulting from the intravenous administration of the tissue extract, and (2) the presence in the extracts of a substance which acts on smooth muscle. Wooldridge[3] made an extensive study covering the action of tissue extracts *in vivo,* and since his time numerous others have worked on the subject. (For literature see Smith[4] and Morse.[5]) The nature and action of the substance affecting the smooth muscle has been studied by Brieger and Uhlenhuth,[6] Dale,[7] Aronson,[8] Popielski,[9] Able and Kubota[10] and Smith.[11]

EXTRACT OF AUTOLYZED LIVER

Fig. 2-*A* and *B* are the records obtained from two animals. The tracings show blood pressure and respiratory response to the injection of the extract of

autolyzed liver tissue. The extract was prepared in a manner similar to the extract of normal tissue, the autolyzed tissue being ground with sand, extracted with its weight of 0.9 per cent sodium chloride solution and filtered. In both tracings it will be noted, that the injection of the extract was accompanied by a marked response in both respiration and blood pressure. The amplitude of respiration becomes greater while the blood pressure shows a sudden fall followed by a rise. The rise in blood pressure is accompanied by a more rapid heart rate, shown in (*A*), and by an arrhythmia shown in (*B*). Such a response suggests the possibility that the toxic substance has a pro-

Fig. 2-*A*.

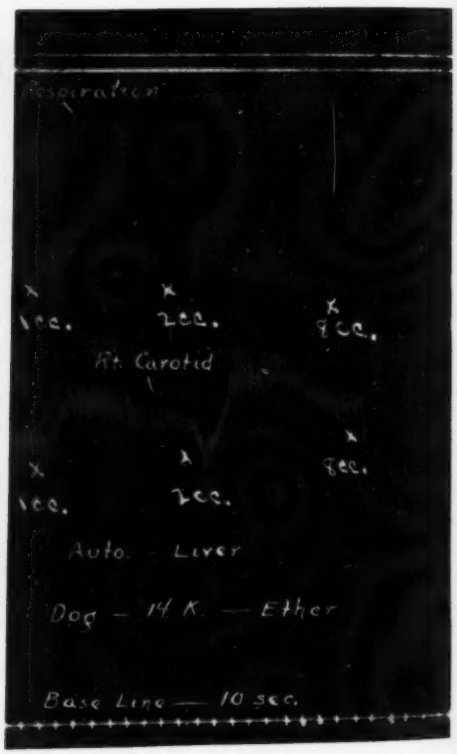

Fig. 2-*B*.

nounced action on circulation, the action being well shown in the latter part of (*B*) where 8 c.c. of the extract caused the sudden death of the animal.

Turck[12] has called attention to the fact that cell necrosis, brought about in various ways, produces results which are highly suggestive of shock symptoms. Delbet[13] has suggested that shock was due to the absorption of toxins arising from disorganized and bruised tissues. Delbet and Karajonopoulos[14] have reported the effects of injecting the product of muscle autolysis into guinea pigs. They found such autolysates very toxic causing an acceleration of respiration, loss of reaction to noise, and death of the animals in a variable time. The authors attributed these effects to disturbances of the nervous system.

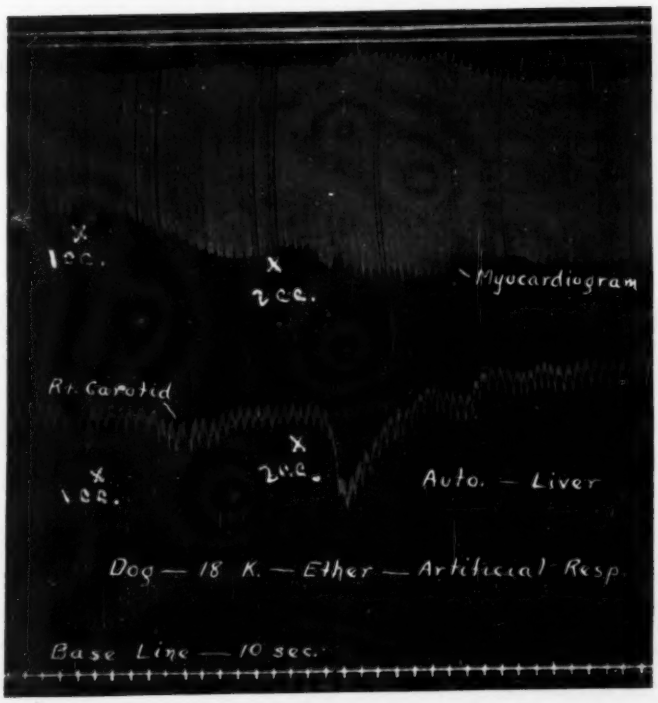
1 cc.
2 cc.
Myocardiogram
Rt. Carotid
1 cc.
2 cc.
Auto. — Liver
Dog — 18 K. — Ether — Artificial Resp.
Base Line — 10 sec.

Fig. 3.

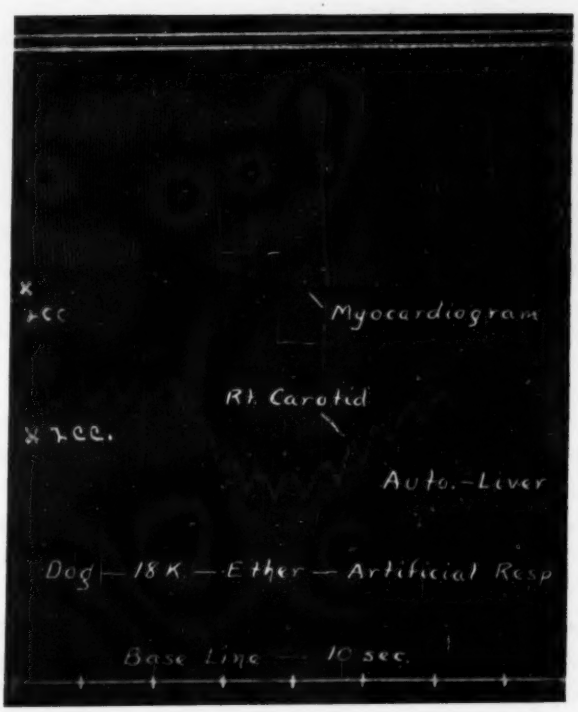
2 cc
Myocardiogram
Rt. Carotid
2 cc.
Auto.-Liver
Dog — 18 K — Ether — Artificial Resp
Base Line — 10 sec.

Fig. 4.

Fig. 3 is a record showing blood pressure record and myocardiogram obtained from a dog weighing 18 kilos, under ether anesthesia and maintained by artificial respiration. The method used for recording the myocardiogram is that described by Jackson.[15] It will be noted that during the fall in blood pressure the amplitude of the heart beat becomes less and apparently there is a slowing of the rate (better shown in Fig. 4.) However, during the rise in blood pressure, following the fall, the amplitude of the beat becomes increased and the rate more rapid.

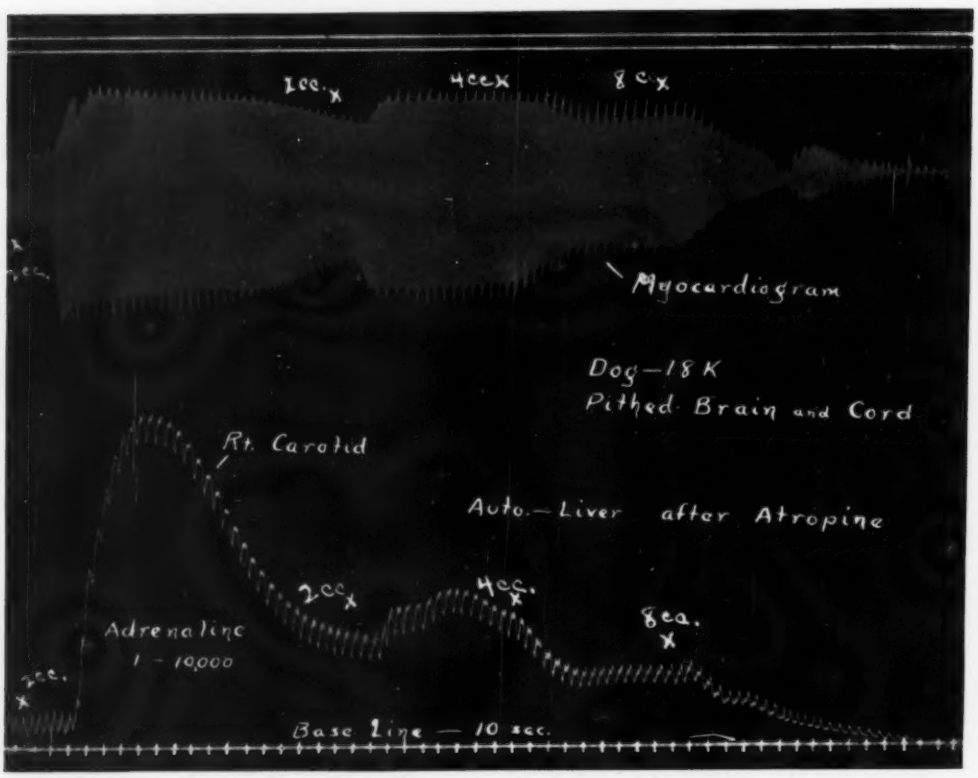

Fig. 5.

Fig. 4 was obtained from the same animal as Fig. 3 with all conditions the same except for an increase in the speed of the drum. It will be observed, that accompanying the fall in blood pressure, the heart beat became less rapid and showed less amplitude. (At the extreme left of the tracing there appears a slowing in the myocardiogram which is not really a slowing but is due to a change in the speed of the drum as shown by the individual heart beats in the blood pressure tracing.) Following the fall in blood pressure the myocardiogram shows an increase of volume and some increase of rate.

In attempting to obtain more definite information as to the point of action of the toxic substance we continued our studies with the use of atropine before the administration of the extract and have also destroyed brain and cord to rule out the possibility of central nervous system response. Fig. 5 is a record show-

ing some of the results obtained by such procedures. The record is one obtained from a dog weighing 18 kilos which had been pithed, brain and cord. He had also received four injections 1 c.c. each of atropine sulphate solution containing 1 mg. per c.c. The record represents blood pressure and myocardiogram. At the beginning of the tracing 2 c.c. of adrenalin (1-10,000) was administered. There is recorded the characteristic rise in blood pressure as well as an increase in the amplitude of the myocardiogram. As the adrenalin action wore off, 2 c.c. of extract of autolytic liver was injected, following which there was a slight

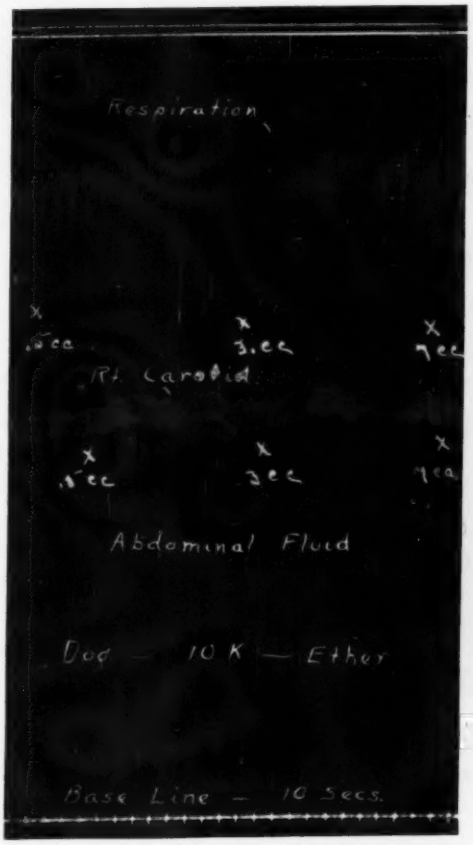

Fig. 6-*A*.

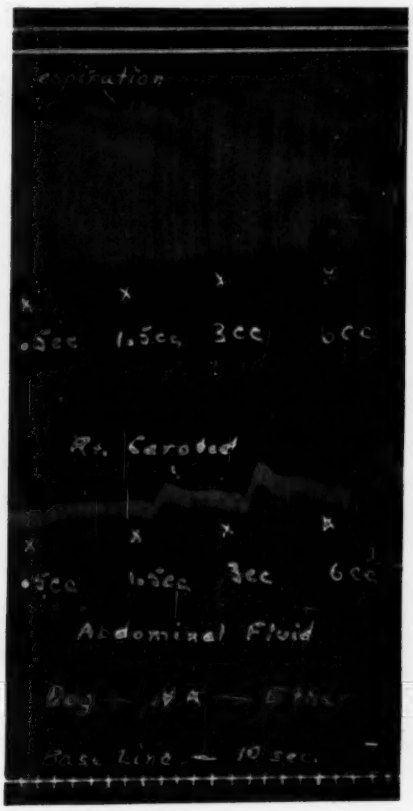

Fig. 6-*B*.

rise in blood pressure as well as an increase in amplitude of heart beat. After conditions became constant 4 c.c. of the extract was administered, and such an amount showed a decrease in myocardial action and a fall in blood pressure. Increasing the dose to 8 c.c. proved fatal to the animal. From this tracing it is evident that the toxic substance acts following the administration of atropine and pithing the brain and cord. The drop in blood pressure is not sudden as in the nontreated intact animal, and this suggests that the toxic substance may not act only on the myocardium but also, to a lesser degree, on the nerve structures. Cannon[2] in his study on shock has made observations with the cord transected and records his observations as follows: "It is only necessary to transect

the spinal cord above the lumbar plexus or to sever all nerves of the limb which is to be injured, in order to disconnect the region from the central nervous system. When this has been done and the denervated muscles are traumatized, events occur similar to those seen after trauma when the nerves are intact. From this evidence it is clear that there is no essential relation between the production of shock and an excessive stimulation of the central nervous system."

ABDOMINAL FLUID

Since the autolyzing liver proved so toxic, we assumed that the abdominal fluid present at the time of death should be quite toxic. We also thought that analysis of the abdominal fluid would probably suggest the nature of the toxic

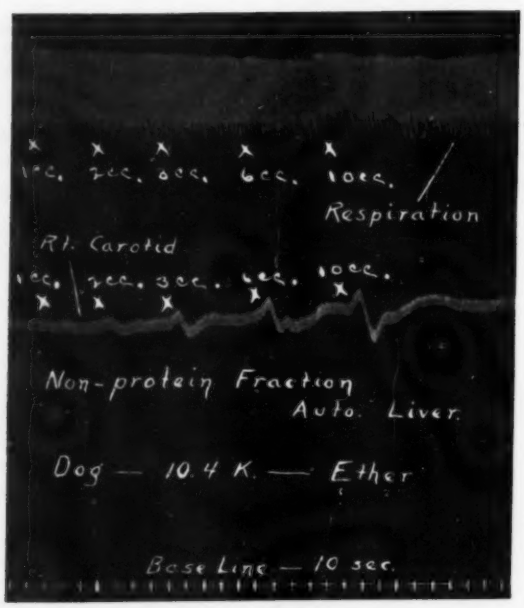

Fig. 7.

substance being absorbed. The following figures were obtained from the analysis of four abdominal fluids:

ANALYSIS OF ABDOMINAL FLUID

Nonprotein nitrogen	52.6	66.6		76.9
Urea nitrogen	33.5	42.0	29.4	35.2
Amino acid nitrogen	12.83	10.85	10.68	14.73
Sugar	35.	33.2	0.	181.8
Uric acid	2.48	2.66	2.4	3.15

It will be noted that all constituents, with the exception of sugar, were above the concentration found in the circulating blood. The variation in sugar is probably related to the time which autolysis has been under progress.

Figs. 6-*A* and *B* are presented to show the relative nontoxic action of the abdominal fluid administered intravenously. The principal action, especially in (*B*), appears to be in increasing blood pressure. The dose was increased to 12 c.c. in (*B*) but due to mechanical difficulties it does not appear on the record.

NONPROTEIN FRACTION

The salt extract of autolytic liver represents quite an indefinite mixture. Therefore we have made a very limited study with the proteins precipitated, using only the filtrate. Briefly: we used 30 c.c. salt extract of the autolyzed liver, added 30 c.c. 5 per cent sodium tungstate, 30 c.c. three-fourths normal sulphuric acid and filtered. Blood was obtained from the animal to be used and 7 c.c. was added to the tungstate filtrate, the whole well shaken and filtered. The filtrate was neutralized to phenolphthalein by adding 15 c.c. of tenth normal sodium hydroxide. Fig. 7 is a record of the blood pressure and respiratory response to the injection of such filtrate. While it is true the response is relatively slight, it should be remembered that the tissue extract has been diluted to approximately four times its original volume during the preparation.

CONCLUSIONS

1. Intravenous administration of the saline extract of autolyzed liver demonstrates the material to be highly toxic.

2. Autolyzed liver tissue contains a toxic substance which apparently is not present in normal liver extract.

3. The toxic substance apparently kills by its action on the circulatory mechanism, such action being principally on the myocardium with possibly a slight action on the nerve structures.

4. Analysis of the abdominal fluid, present at the death accompanying autolysis, shows the fluid to be higher in nonprotein nitrogen, urea, uric acid, and amino acid than the circulating blood, but usually lower in sugar.

5. The abdominal fluid is relatively much less toxic than the autolyzed liver.

6. After a limited study it appears that the toxic material is partially if not wholly present in the nonprotein nitrogen fraction.

7. There appears to be a relation between our observations and those reported by Cannon in his studies on "Wound Shock."

REFERENCES

[1]Mason and Davidson: Jour. Lab. and Clin. Med. (In press.)
[2]Cannon: Arch. Surg., 1922, iv, 1.
[3]Wooldridge on the Chemistry of the Blood and other Scientific Papers, London, 1893.
[4]Smith: Jour. Lab. and Clin. Med., 1919, iv, 517.
[5]Morse: Jour. Lab. and Clin. Med., 1919, v, 319.
[6]Brieger and Uhlenhuth: Deutsch med. Wchnschr., 1898, xxiv, 163.
[7]Dale and Coworkers: Jour. Physiol., 1910, xl, 503; ibid., 1910, xli, 318; ibid., 1911, xli, 499; ibid., 1918, lii, 110; ibid., 1919, lii, 355.
[8]Aronson: Berl. klin. Wchnschr., 1913, 1, 253.
[9]Popielski: Bull. internat. Acad. d. sc. de Cracovie, 1912, p. 1157 (Chem. Abstr., viii, 1463).
[10]Able and Kubota: Jour. Pharmacol. and Exper. Therap., 1919, xiii, 283.
[11]Smith:[4]
[12]Turck: Med. Rec., 1919, xcv, 472; ibid., 1918, xciii, 927.
[13]Delbet: Bull. et mém. Soc. de chir. de Par., 1917, xliii, 2121.
[14]Delbet and Karajonopoulos: Bull. Acad. de méd., 1918, lxxx, 14.
[15]Jackson: Exper. Pharmacol., St. Louis, 1917, C. V. Mosby Co., p. 149.

STUDIES WITH B. ACIDOPHILUS AND B. BULGARICUS

I. Preliminary Attempts at Implantation of B. Bulgaricus*

By Nicholas Kopeloff, Ph.D., and Philip Beerman, Ward's Island, N. Y.

THE failure of B. bulgaricus in the United States and the subsequent success of B. acidophilus therapy, raises many interesting clinical and bacteriologic problems. Herter and Kendall,[1] in 1908, were unable to establish the predominance of B. bulgaricus in the ileocecal region or large intestine of monkeys after exclusive feeding of bacillac milk. Rahe[2] demonstrated that while an apparently limited survival of B. bulgaricus could be obtained in the upper intestine of monkeys, nevertheless, this organism could not become adapted to the human lower intestine, despite its occasional recovery after its administration has been stopped. The comprehensive experiments of Rettger and Cheplin[3] finally sealed the fate of B. bulgaricus and stimulated further interest in B. acidophilus.

It should be noted, however, that conclusive as the aforementioned data appear, Rahe's work was based on four human subjects and Rettger and Cheplin's on four human subjects. In view of the favorable implantation of B. bulgaricus reported by Cohendy[4] and Belonovsky,[5] as well as the continued use of Metchnikoff's B. bulgaricus therapy in France, there would still seem to be a place for further studies on a larger scale. This is particularly true since the differentiation of B. bulgaricus from B. acidophilus remains a vexing problem.

In morphology and cultural characteristics these closely related organisms are similar. Heinemann and Hefferan[6] claimed that B. bulgaricus was widely distributed in nature; in feces from cows, horses, and man, in human fluids, etc., and that "some bacilli isolated from various fermented milks, so-called acidophile bacteria found in the intestinal tract of man, Boas-Oppler bacillus, B. panis fermentati, St. Lebenis, Leptothrix bucalis and possibly B. bifidus of Tissier are identical with B. bulgaricus." On the other hand, Rahe[7] established that B. acidophilus ferments maltose, while B. bulgaricus does not. This important distinction has been accepted by most of the investigators in this field; Rettger and Cheplin,[3] Cannon[8] and others. Kulp and Rettger[9] in a comparative study have noted many variations in fermentative power, although they state that "the action upon maltose, sucrose and levulose appears to furnish a valuable means of separating strains of these two groups. In addition to being unlike in their fermentative action upon the three sugars mentioned, they differ in another very important respect, namely, in that L. acidophilus can and does develop in the intestine of man and animals

*Department of Bacteriology, Psychiatric Institute of the New York State Hospitals, Ward's Island, N. Y.
Received for publication, January 4, 1925.

whereas, L. bulgaricus is unable to do so.'' Recently, Cruickshank and Berry[10] have shown that of 52 strains of B. acidophilus, isolated from human feces, 44 did not ferment maltose and 4 did not ferment glucose.

The present study grew out of an investigation which one of us carried on at the Pasteur Institute, in Paris, in the Department of Physiological Chemistry under G. Bertrand and this opportunity is taken to express my deep appreciation of the courtesies extended. While data there obtained, using rats, have not yet been published, in the main they corroborated the findings of Rahe[2] to which reference has been previously made. However, they were equivocal enough to require further investigation, particularly as regards the implantation of B. bulgaricus in the human intestine. Therefore, a group of 12 women (psychotic) were selected for fecal examination and administration of B. bulgaricus milk.

The procedure was as follows: Two or more fecal examinations were made on about 35 women (psychotic) to select a group in which no colonies showing the typical morphology of B. acidophilus or B. bulgaricus were found on whey agar plates. Seven constipated and five nonconstipated subjects were thus selected for administration of milk fermented by B. bulgaricus. The cultures used for this purpose were: A strain called the Pasteur Institute bacillus corresponding to Metchnikoff's original culture, kindly furnished by Legros of the Pasteur Institute, Paris; and two strains used by Kulp and Rettger, labelled B-3 and B-4 respectively, which Kulp of Yale University was kind enough to furnish. The technic of preparing milk, plating, etc., was the same as described by us in employing B. acidophilis milk, except that the counts averaged about 50,000,000 viable organisms per cubic centimeter.[11]

The twelve subjects were arranged in groups of four; two constipated, and two nonconstipated, to receive milk fermented by the same strain of B. bulgaricus (an exception to this plan was the substitution of a constipated subject, M. Fy. for a nonconstipated one). The administration of one liter of bulgaricus milk per day in addition to the regular hospital diet was carried on with the Pasteur Institute bacillus for fifteen days, with B-3 for twelve days and with B-4 for ten days. Three fecal examinations at three-day intervals were made during treatment and two after treatment. Counts were made from the dilutions, 1:100,000 and 1:1,000,000.

During the treatment, there were only two subjects showing B. bulgaricus. One subject, T. Co., had 20 per cent after one week of treatment and 10 per cent after thirteen days of treatment. Subsequently no B. bulgaricus were found in her feces. The other subject, D. Pr., showed 50 per cent of B. bulgaricus, after one week of treatment and 60 per cent after ten days. The day, after treatment had been discontinued, this was the only subject to have B. bulgaricus present. There were 80 per cent found. However, they disappeared in the following seven days, when no B. bulgaricus was recovered. It is significant that both these subjects were nonconstipated to begin with. No change was noted in the color, character, or consistency of the feces from these subjects although daily observations were recorded.

One might be led to conclude from these data that they corroborated previous investigations which established that B. bulgaricus is not implanted

in the human intestine. However, such a conclusion might be premature without first considering the dosage. It has been stated that the fermented milk averaged about 50,000,000 viable organisms per cubic centimeter. Elsewhere[12] the importance of the number of viable organisms per cubic centimeter of milk has been stressed. Suffice it to say that any conclusion to be derived from the foregoing data should be considered in the light of the number of viable organisms present. It is conceivable that an implantation of B. bulgaricus, might occur with larger amounts such as 200,000,000 per cubic centimeter, as previously employed by us in B. acidophilus milk. The plan of this experiment, of which this represents but the first step, calls for a repetition of the administration of milk fermented by B. bulgaricus which will contain larger numbers of viable organisms. Finally, after discontinuing treatment with B. bulgaricus, those subjects who have not had any B. bulgaricus in their feces will be given B. acidophilus milk. In this way further evidence may be added to the crucial question of whether B. bulgaricus can be implanted in the human intestine.

It is a privilege to acknowledge our indebtedness to George H. Kirby, Director of the Psychiatric Institute and to Helen Langner of the Manhattan State Hospital.

SUMMARY

Subject to the limitations of the data, it has been shown in this preliminary study, that:

1. Of 12 human subjects receiving 1 liter of B. bulgaricus milk for from ten to fifteen days, only 2 revealed the presence of B. bulgaricus in the feces. In one of these subjects the number of B. bulgaricus present was so small as to be negligible.

2. Since the bacterial content of the milk fed was only 50,000,000 per cubic centimeter, it is believed that further corroboration of the failure to implant B. bulgaricus is required, when using the same dosage of milk containing about 200,000,000 per cubic centimeter, as is recommended in the feeding of B. acidophilus.

REFERENCES

[1]Herter, C. A., and Kendall, A. I.: An Observation on the Fate of B. Bulgaricus (in Bacillac) in the Digestive Tract of a Monkey, Jour. Biol. Chem., 1908, v, 293-302.

[2]Rahe, A. H.: A Study of the So-Called Implantation of the Bacillus Bulgaricus, Jour. Infect. Dis., 1915, xvi, 210-220.

[3]Rettger, L. F., and Cheplin, H. A.: A Treatise on the Transformation of the Intestinal Flora with Special Reference to the Implantation of Bacillus Acidophilus, Yale University Press, 1921.

[4]Cohendy, M.: Description d'un ferment lactique puissant capable de s'acclimater dans l'intestin de l'homme, Compt. rend. Soc. de biol., 1906, lx, 558-560.

[5]Belonovsky, G. D.: Sur la prolongation de la vitalite' du Bacille Bulgare, Compt. rend. Soc. de biol., 1913, lxxv, 374-375.

[6]Heinemann, P. G., and Hefferan, M.: A Study of B. Bulgaricus, Jour. Infect. Dis., 1909, vi, 304-318.

[7]Rahe, A. H.: An Investigation into the Fermentative Activities of the Aciduric Bacteria, Jour. Infect. Dis., 1914, xv, 141-150.

[8]Cannon, P. R.: A Biologic Study of Aciduric Bacteria, Jour. Infect. Dis., 1924, xxxiv, 227-238.

[9]Kulp, W. L., and Rettger, L. F.: Comparative Study of Lactobacillus Acidophilus and Lactobacillus Bulgaricus, Jour. Bacteriol. 1924, ix, 357-394.

[10]Cruickshank, J., and Berry, D. W.: A Study of B. Acidophilus in Human Feces, Brit. Med. Jour., Nov. 22, 1924, No. 3334, 944-948.
[11]Kopeloff, N., and Cheney, C. O.: Studies on the Therapeutic Effect of Bacillus Acidophilus Milk and Lactose, Jour. Am. Med. Assn., 1922, lxxix, 609-611.
[12]Kopeloff, N.: Clinical Results Obtained with Bacillus Acidophilus, Arch. Int. Med., 1924, xxxiii, 47-54.

PHYSICOCHEMICAL ASPECTS OF HEMOLYSIS

III. A Further Study of Some of the Factors Involved*

By Reed Rockwood, M.D., and Edward C. Mason, M.D., Rochester, Minnesota

In a previous paper on this subject, we[5] have called attention to the fact that the various reactions of the red cell favor the conclusion that it is essentially a mass of colloidal components, the chief colloids from the standpoint of hemolysis being the stroma and the hemoglobin. We further expressed the belief that the stroma-hemoglobin union (red cell) was of a physical nature, and possessed many of the characteristics of an adsorption phenomenon. Our studies with such combination led us to summarize our interpretation as follows: "All agents which tend to change the red cell from a state of (1) small mass, (2) low hydration capacity, (3) maximal cohesion, and (4) relative electrical neutrality to a state of (1) large mass, (2) increased hydration capacity, (3) decreased cohesion (which is accompanied by decreased surface tension), and (4) away from the isoelectric point, do to the same degree promote hemolysis."

In the present study we have extended our observations to include cell volume changes in the presence of various hemolytic agents, and viscosity changes during biologic hemolysis; that is, during the reaction of cells, amboceptor, and complement.

THE RELATION OF CELL VOLUME TO HEMOLYSIS

The literature on this subject exhibits somewhat conflicting data. Fischer, on theoretic grounds, believes that volume changes and loss of hemoglobin are separate processes which only happen to parallel each other at times. He believes that hemolysis by hypotonic solutions, acids and alkalis is probably accompanied by some swelling of the cell colloids, but not of other forms. Ponder, by the use of a formula which determines the approximate cell volume from measurements of the diameter, concludes that the volume of cells hemolyzed by hypotonic solutions was about 448 cubic micra at the time of hemolysis, while with saponin it was 131 cubic micra, and with sodium taurocholate 187 cubic micra, the normal cell having a volume of about 70 cubic micra. In biologic hemolysis (cells, amboceptor, and complement) he noted no change. Von Knaffl-Lenz made hematocrit readings on cells and found no change in cell

*Work done in the Division of medicine, Mayo Clinic, Rochester, Minnesota.
Received for publication, November 8, 1924.

volume on the addition of saponin. The cell volume decreased or was not altered with the use of ethyl alcohol, ether, urethane, salicylamide, benzamide, camphor or urea in small quantities, on account of the loss of water, and thus these substances opposed hemolysis by hypotonic solutions. In higher concentrations they caused swelling. Brahmachari, in hematocrit studies, found no change in cell volume on the addition of amboceptor. We have performed the following experiments with the hope of adding to our knowledge of the relation between cell volume and hemolysis.

METHOD OF STUDY

Fresh washed beef cells were placed in graduated centrifuge tubes with different amounts of the hemolytic agent, and the volume made up to 10 c.c.

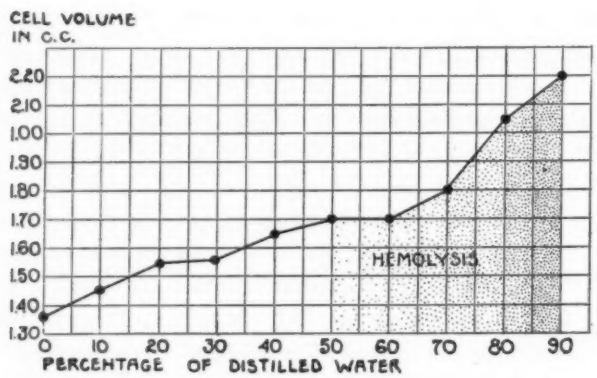

Fig. 1.—Hemolysis by distilled water.

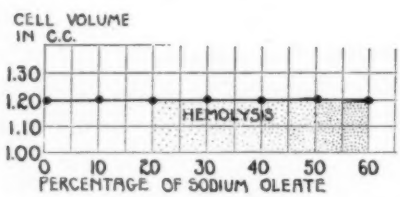

Fig. 2.—Hemolysis by sodium oleate. 1:1000.

with isotonic salt solution. They were then centrifugalized at constant speed at room temperature (25° to 30°) for ten minutes at about 2000 revolutions, the tubes in each group of tests being in the centrifuge at the same time. The volume of the cells in the bottom of each tube was then read. The concentrations of the hemolytic agents used were designed to cause, at the most, a slight hemolysis. A greater degree of hemolysis would destroy too many cells, thus decreasing the cell volume so that no results of value could be obtained. Of course it is obvious that this method measures the alterations in the cell mass only, not the change in the individual cell which occurs as it hemolyzes.

RESULTS

The results of this method are exemplified in Fig. 1, which shows the marked increase in cell volume during hemolysis by distilled water in varying

proportions in isotonic salt solution. On the other hand, amboceptor and complement, 1:1000 sodium oleate, and 1:1000 saponin in isotonic salt solution showed no significant variation. Fig. 2 is representative of this group.

Hemolysis by autolysis, that is, by standing for several hours, can be prevented by the addition of hypertonic sodium chloride. This has been discussed by one of us in a previous paper. Figs. 3 and 4 show the changes in cell volume which accompany this phenomenon. In the protected zone the hypertonic salt solution causes a decrease in volume, while the autolyzing cells and those hem-

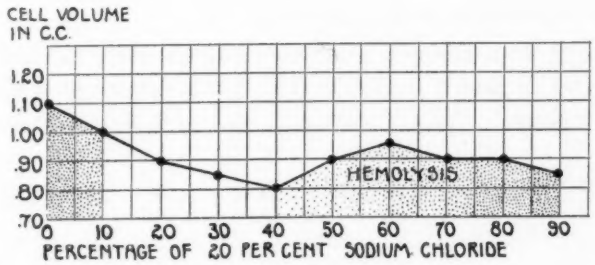

Fig. 3.—Inhibition of autolytic hemolysis by hypertonic salt solution. Time, sixteen hours.

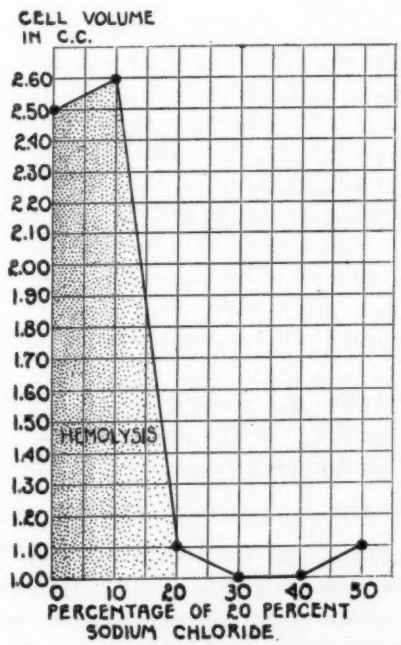

Fig. 4.—Inhibition of autolytic hemolysis by hypertonic salt solution. Time, eighteen hours.

olyzed by the highest concentrations of sodium chloride show an increase in volume.

Variable results are secured with the use of hydrochloric acid. In some cases there is a swelling, the most marked increase in total volume of unhemolyzed cells and stroma being 44 per cent. More frequently only a slight swelling was observed; sometimes none at all, hemolysis still occurring. This would tend to confirm Fischer's general concept that cell swelling and hemolysis are

independent processes. In using sodium hydroxide in sufficient concentration to cause beginning hemolysis, no significant alteration in cell volume was noted.

The P_H of the solutions was determined colorimetrically, after pipetting off the supernatant fluid, by matching them with the Clark and Lubs standards. Owing to hemolysis, the reading in some tubes was difficult, and can only be considered as approximate. Because of the lack of buffer, the solution could not be diluted to decrease the color of the hemoglobin. In the experiments with phosphate buffer, dilution could be made, and hence the readings on buffered solutions with hemolysis are much more accurate. Tables I and II are typical of the results obtained with acid and alkali.

An experiment with isotonic phosphate buffers (M/7 KH_2PO_4 and M/8.8 Na_2HPO_4, $2H_2O$) is shown in Table III. Such experiments show no marked variation in cell volume. We are unable to interpret the slight tendency for the

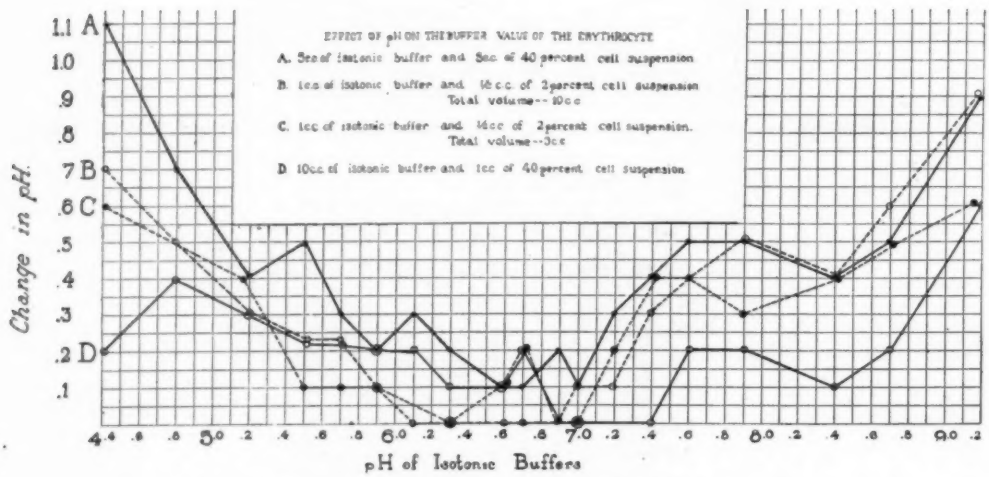
EFFECT OF pH ON THE BUFFER VALUE OF THE ERYTHROCYTE
A. 5cc of isotonic buffer and 5cc of 40 percent cell suspension
B. 1cc of isotonic buffer and 1/2 c.c. of 2 percent cell suspension. Total volume--10c.c
C. 1cc of isotonic buffer and 1/4cc of 2 percent cell suspension. Total volume--3cc
D 10cc of isotonic buffer and 1cc of 40 percent cell suspension
Change in pH
pH of Isotonic Buffers

Fig. 5.—Effect of P_H on the buffer value of the erythrocyte.

cell volume to decrease toward the alkaline end of the range, but we have seen it in other similar experiments. It will be noted that hemolysis increases with acidity, alkalinity, and dilution of the salt.

THE BUFFER VALUE OF THE ERYTHROCYTE AND ITS RELATION TO HEMOLYSIS

It may be noted in Fig. 5 that the addition of cells has considerable buffering effect on the phosphate solutions; this is not uniform at each P_H; the amount of buffering depending on the P_H of each solution, the most marked effect being at the extreme acid and alkaline sides of the range. This observation has been confirmed in a number of instances. Thus, the experiment in our original paper on the effect of acidity on hemolysis has been repeated, and the actual P_H in each tube has been determined colorimetrically after centrifugalizing. Isotonic phosphate buffers were used in this case, and the general results as far as hemolysis is concerned were the same as those previously reported. Small amounts of cells can apparently also exercise a considerable buffer power. Thus 1 c.c. of isotonic buffer and 0.5 c.c. of 2 per cent sheep cells in a total of 3 c.c. of fluid (0.3 per cent suspension) show a marked buffer action in extremely acid and

TABLE I

EFFECT OF ONE-FIFTIETH NORMAL HYDROCHLORIC ACID ON CELL VOLUME

TUBES	1	2	3	4	5	6	7	8
Isotonic sodium chloride, c.c.	5.0	4.5	4.0	3.5	3.0	2.5	2.0	1.5
Acid	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5
Hemolysis	0	0	*	**	**	***	***	+
Volume	2.6	2.6	2.5	2.6	2.6	2.6	2.6	2.7
P_H	7.2	7.4	6.8	6.7	6.6	6.4	6.0	6.0

*Very faint trace. **Faint trace. ***Trace. +Considerable hemolysis.

TABLE II

EFFECT OF ONE-TWENTIETH NORMAL SODIUM HYDROXIDE ON CELL VOLUME

TUBES	1	2	3	4	5	6	7	8
Isotonic sodium chloride, c.c.	5.0	3.0	2.5	2.0	1.5	1.0	0.5	0
Alkali	0	2.0	2.5	3.0	3.5	4.0	4.5	5.0
Hemolysis	0	0	0	0	*	*	**	+
Volume	2.3	2.2	2.2	2.2	2.1	2.2	2.3	2.3
P_H	7.2	10.0 to 10.5	12.1 to 12.7	12.1 to 12.7	12.1 to 12.7	12.1 to 12.7	Greater than 12.7	

*Faint trace. **Trace. +Considerable hemolysis.

alkaline ranges. This is the amount of cells and volume of fluid used in the Kolmer technic for the Wassermann reaction. These experiments were repeated, making the total volume 10 c.c.; the resulting curve was the same. These four curves of buffer value are shown in Fig. 5.

It is obvious from these curves that the maximal buffering power of the cell is not exerted, except at the extremes of acidity and alkalinity. This means that in the buffering which occurs at ordinary ranges all the cell buffers are not

TABLE III

EFFECT OF ISOTONIC PHOSPHATE BUFFERS ON CELL VOLUME AND HEMOLYSIS

Each Tube contains 5 c.c. of buffer and 5 c.c. of cell suspension

P_H OF BUFFER	4.4	4.8	5.2	5.5	5.7	5.9	6.1	6.3	6.6	6.9	7.2	7.4	7.6	7.9	8.4	9.2
Hemolysis	*	**	***	0	0	0	0	0	0	0	0	0	0	0	0	0
Cell volume	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.0	2.0	1.9	1.9	1.8	1.8	1.8	1.8
Correct P_H	5.5	5.5	5.6	6.0	6.0	6.1	6.4	6.5	6.5	6.7	6.9	7.0	7.1	7.4	8.0	8.3
1 C.C. OF DISTILLED WATER ADDED																
Hemolysis	+	+	+	†	†	*	*	**	***	0	0	0	0	0	0	0
Cell volume	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.0	2.0	1.9	1.8	1.8	1.8	1.8	1.8
1 C.C. OF DISTILLED WATER ADDED																
Hemolysis	2+	2+	2+	+	+	†	†	†	*	**	0	0	0	0	0	**
Cell volume	1.8	1.8	1.8	2.0	1.9	2.1	2.1	2.1	2.1	2.1	2.0	1.9	1.9	1.9	1.9	1.9
1 C.C. OF DISTILLED WATER ADDED																
Hemolysis	3+	3+	3+	2+	2+	+	+	+	†	*	**	**	0	0	0	**
Cell volume	1.4	1.4	1.4	1.6	1.6	1.8	1.8	1.9	2.0	2.0	2.0	1.9	1.9	1.9	1.9	1.9
1 C.C. OF DISTILLED WATER ADDED																
Hemolysis	4+	4+	4+	3+	3+	2+	2+	2+	+	†	†	†	*	**	**	*
Cell volume	0.7	0.7	0.7	1.0	1.0	1.3	1.4	1.4	1.8	2.0	2.1	2.0	2.0	2.0	2.0	1.9

*Faint trace. **Very faint trace. ***Slightest possible trace. †Trace.
+Considerable hemolysis.

available, while another source can be made effective as the acidity or alkalinity increases. The most important cell buffers are the bicarbonate and cell proteins. The cell phosphate is apparently of little importance. It would seem most likely that the proteins of the cell are responsible for the peculiarities of the buffer curves, and form the final reserve. The relative importance of hemoglobin and stroma protein cannot be determined. However, Van Slyke, Hastings, and Neill have calculated that for oxygenated blood the hemoglobin was responsible on an average, for 76.0 per cent of the total buffer value, and the bicarbonate for 6.9 per cent of it. In reduced hemoglobin the figures were 73.3 per cent and 9.0 per cent respectively. This work involves the assumption that the confinement of hemoglobin within the red cells does not alter its buffer value in the system. The relative preponderance in mass of hemoglobin over protein in the stroma suggests also its predominant importance.

The similarity of these curves of buffer action to the curves showing the action of acid and alkali in causing hemolysis suggests the possibility of a causal connection between the two. It might be postulated that the change which takes place in the hemoglobin, and possibly also in the stroma protein in an attempt at buffering the solution so alters these proteins that the hemoglobin stroma union is rendered unstable. It should be recalled in this connection that it would be possible by increasing the acidity of the solution to carry these proteins past their isoelectric points, and thus reverse their charges. This could not be done by putting them in more alkaline solutions, since they are normally on the alkaline side of their isoelectric points. But again it has been shown that the cell is much more easily hemolyzed by acids than by alkalis.

VISCOSITY AND BIOLOGIC HEMOLYSIS

Assuming that the red cells, amboceptor, and complement unite during the course of the biologic hemolysis, it seemed reasonable to us that the resulting state should represent fewer particles, and consequently should have less viscosity. On the other hand, an increase in the hydration capacity of such a system would lead to an increase in viscosity. Therefore, it is evident that the resulting viscosity would probably depend on the balance of these two phenomena.

Viscosity determinations were made on each of the components of the biologic hemolytic system, separately, and combined. The apparatus used was that designed by Hess, which has certain advantages, owing to the horizontal position of the capillaries which eliminates the influence of specific gravity, and, since the water and the colloid are tested simultaneously, there is no need for temperature corrections. The differences between combinations of the three reagents and complement alone were so slight, however, as to make it impossible to draw any definite conclusions.

THE HEMOLYSIS OF AGAR HEMOGLOBIN COMBINATIONS

In our previous paper, we discussed the relationship of hemoglobin and kaolin combinations to their union and dissociation. We have also obtained similar results by substituting an emulsoid, such as agar, for a suspensoid like kaolin.

An agar jelly was made of 12.5 gm. of agar to 1000 c.c. of 0.85 per cent

sodium chloride. The agar was cooled to just above the point of solidification, and to each 100 c.c. was added 20 c.c. of phosphate buffer of known P_H, and 10 c.c. of hemoglobin solution made by hemolyzing washed red blood corpuscles in distilled water. After the agar-hemoglobin mixtures had cooled, they were ground in a mortar at room temperature as finely as possible, and allowed to stand overnight. They were then washed in isotonic sodium chloride solution

TABLE IV

"HEMOLYSIS" OF HEMOGLOBIN-AGAR COMPLEX

P_H OF SOLUTION IN TUBE	P_H OF AGAR PARTICLES					
	6.2	6.6	6.9	7.3	8.0	9.2
4.4	±	±	±	++	++++	++++
5.5	±	±	++	+++	+++	++++
6.2	++	+	++	++	+++	++++
6.6	+++	±	+	++	+++	+++
6.9	++	+	+	+	++	++
7.3	±	+++	+++	+	+	++
8.0	0	+++	++	0	±	+
9.2	+++	++	++	++	+	+

+Indicates degree of hemolysis.

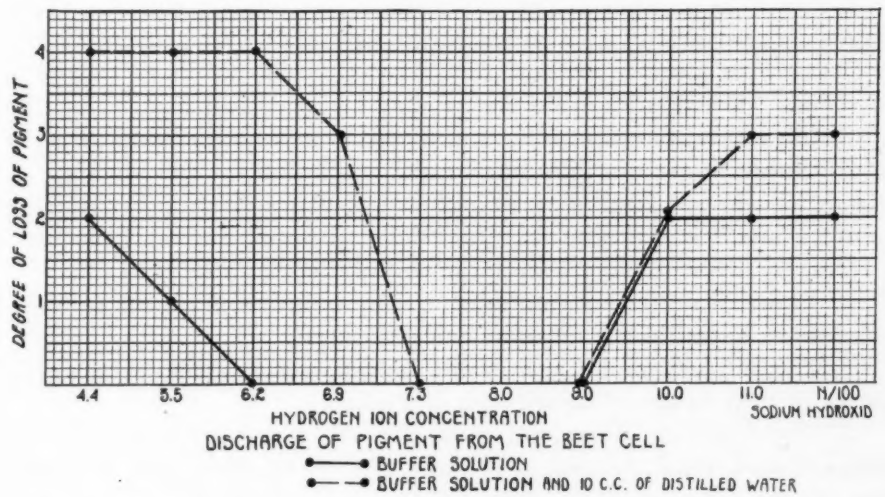

Fig. 6.—Hydrogen-ion concentration. Discharge of pigment from the beet cell.

until the solution was nearly clear. Two grams of agar particles were then added to 20 c.c. of buffer phosphate solution in a test tube, and the process of hemolysis observed. The amount of hemoglobin released was graded approximately by matching the tubes from 1 to 4. The two buffers of highest acidity, that is, P_H 4.4, and 5.5, were acid enough to change the hemoglobin to dark brown. There was no discharge of pigment, and hence these buffers could not be used.

It will be seen from the results in Table IV that there is a distinct tendency for the hemoglobin to separate from the acid mixtures when they are placed in alkaline solutions, and from the extremely alkaline mixtures when placed in acid solutions. The mixtures which were originally near the neutral point, like the hemoglobin-kaolin mixtures previously described, and the red blood corpuscles themselves "hemolyze" in the presence of both acid and alkali. Fischer's

experiments with combinations of fibrin and carmin are of great interest in this connection.

These findings are somewhat analogous to those of Kosaka and Seki with the cells themselves. They believe that the corpuscles which were most strongly charged negatively were most easily dissolved in acid, and that the least powerfully charged dissolved most readily in alkali.

The results in our experiments apply to other cells as well as to the red blood corpuscles. Thus, Fig. 6 shows the relation of acidity and salt concentration to the discharge of the pigment from the cell of the red beet. The curves are strikingly similar to those observed in the case of hemolysis and the dissociation of hemoglobin-colloid complexes. They were secured by placing cubes of beet in buffer phosphate solution and diluting the mixtures up to varying degrees. The exact location of the pigment in the beet cell has not been carefully worked out. The best information appears to be that some of it is present in the vacuoles, while another portion may be bound to other constituents of the protoplasm, or even present in crystalline form.

CONCLUSIONS

1. Under the conditions of the experiment described, hemolysis by hypotonic solutions is accompanied by swelling of the cell mass, while hemolysis by alkali, complement and amboceptor, saponin and sodium oleate shows no change in the cell volume. Hemolysis by acid may or may not be accompanied by swelling.

2. The cells have considerable buffer power; it is not the same at every P_H, but is greatest at the extremes of acidity and alkalinity. This suggests a combination of acid and alkali with an additional buffer substance in these parts of the range, and it is suggested that this combination may play an important part in rendering the hemoglobin-stroma union unstable in these zones.

3. Viscosity determinations on the components of the biologic hemolytic system show no significant change during hemolysis.

4. The hemoglobin and agar mixtures show a certain similarity to the corpuscles in their behavior toward acid and alkali.

5. The discharge of pigment from the cell of the red beet under the influence of acid, alkali, and salt is in many respects similar to loss of hemoglobin from the red cell or from the hemoglobin-colloid complexus.

REFERENCES

[1]Brahmachari, U. N.: An Investigation into the Physico-chemical Mechanism of Hemolysis by Specific Haemolysins, Biochem. Jour., 1913, vii, 562-567.

[2]Fischer, M. H.: Edema and Nephritis. A Critical Experimental and Clinical Study of the Physiology and Pathology of Water Absorption in the Living Organism, New York, 1915, Wiley and Sons, pp. 695.

[3]Knaffl-Lenz, v. E.: Über die colloidchemischen Vorgänge bei der Hämolyse, Arch. f. d. ges. Physiol., 1918, clxxi, 51-65.

[4]Kosaka, K., and Seki, M.: Electric Charges of the Red Blood Corpuscles, Chem. Abstr., 1923, xvii, 1825.

[5]Mason, E. C., and Rockwood, Reed: Some Physicochemical Aspects of Hemolysis, Jour. Lab. and Clin. Med., 1924, x, 10-18.

[6]Ponder, E.: Alterations in the Diameters of the Erythrocytes During Hemolysis, Proc. Roy. Soc. Med., London, 1922, s. B., 102-108.

[7]Rockwood, Reed: Physicochemical Aspects of Hemolysis. II. An Ultramicroscopic Study of Hemolysis, Jour. Lab. and Clin. Med., 1924, x, 19-31.

[8]Slyke, van D. D., Hastings, A. B., and Neill, J. M.: Studies of Gas and Electrolyte Equilibria in the Blood. IV. The Effect of Oxygenation and Reduction on the Bicarbonate Content and Buffer Value of Blood, Jour. Biol. Chem., 1922, liv, 507-526.

LABORATORY METHODS

A METHOD FOR INSTANTANEOUS PHOTOMICROGRAPHY OF THE SKIN CAPILLARIES*

By Charles Sheard, Ph.D., and George E. Brown, M.D.,
Rochester, Minnesota

THE clinical investigation of the capillaries of the skin has opened up a new field of vascular research which may be of value both from physiologic and clinical standpoints. In health and in certain diseases these studies have enlarged our knowledge of the disturbances which take place in this important part of the vascular field. Capillary studies have been directed toward defining form, size, tonus, flow, and reaction to stimuli. While many of these studies relative to size have been of a quantitative nature, employing the eyepiece micrometer, still considerable error has existed, and permanent and accurate records have been lacking. This has been true particularly in obtaining accurate data relative to the capillary caliber. The quantitative determinations of the caliber in these vessels may be useful as an index of capillary tonus and permeability, for Krogh has shown that permeability and dilatation of the capillaries are directly related. The capillary response to various agents, as well as data on measurements of the visible capillary loop or loops, can be more accurately determined from photographic records. The number of functioning capillaries for each unit area can also be made a matter of record, thus securing a quantitative index of the metabolic activity of the skin. The determination of the capillary flow is likewise of importance. It is possible that a method may be worked out for determining this more accurately than the present methods permit, whereby the passage of the leucocyte can be imaged, timed, and measured by means of multiple photographs.

DIFFICULTIES IN THE PHOTOMICROGRAPHY OF THE SKIN VESSELS

Several workers have attempted to utilize a practical method of photographing the vessels of the skin, but there have been certain fundamental errors and difficulties involved which have prevented their further application. Little light is needed to make the capillaries microscopically visible to the observing eye. The problem from the photographic standpoint, however, is much more difficult, owing chiefly to (1) inability to get sufficient light reflected from the capillaries and returned to the photographic plate or film so that photomicrographs can be obtained in a fraction of a second; (2) with longer exposures, of the order of a few seconds, the difficulty of preventing mechanical movements of the finger or apparatus, and eliminating the action

*Received for publication, December 26, 1924.
From the Mayo Clinic, Rochester, Minnesota.

of heat and actinic rays on the vessels; (3) the impossibility of preventing slight rhythmic movements due to arterial pulsations and (4) the difficulty of coordinating the eye and plate focus.

Preliminary experiments* demonstrated the inadvisability of endeavoring to overcome difficulties (2) and (3), and indicated that success in instantaneous photomicrography of the capillaries would result only through the use of intense external illumination, so filtered as to remove heat radiation, and directed at such an angle as to throw the light reflected by the surface of the skin out of the path of the light reflected by the capillaries and, in turn, trans-

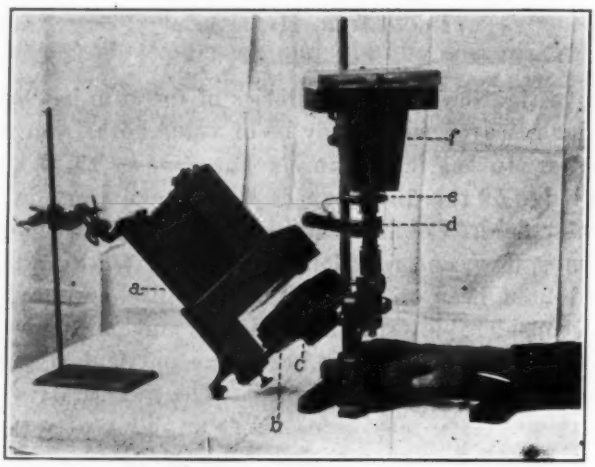

Fig. 1.—Showing ensemble of photographic apparatus. Arc lamp, *a*; water-copper chloride filter, *b*; lens system, *c*; demonstration eyepiece, *d*; shutter, *e*; camera, *f*.

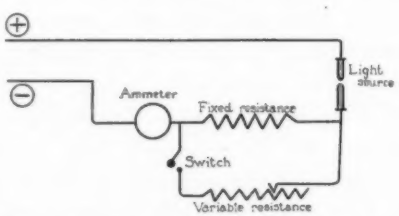

Fig. 2.—Diagrammatic sketch of electrical circuit. Ammeter; fixed resistance; variable resistance to throw in parallel with fixed resistance; switch; and carbon arc.

mitted to the photographic plate or film. Other difficulties also arise by reason of the character of the oil used, the texture of the skin, the presence of moisture under the oil film caused by sweating, the condition of the cuticle and the slight readjustments of focus necessary under the higher illumination used when photographing.

METHOD

The essential pieces of apparatus are shown in Fig. 1. All electrical connections and other similar details, familiar to electricians, are omitted. Fig. 2 gives a diagrammatic sketch showing the details of the electrical circuit.

*Sheard, Charles: Instantaneous Photomicrography of the Skin Capillaries in the Living Human Body, Science, 1924, lx, 409-410.

The light source is a direct current arc (Fig. 1, a) running at 5 amperes or thereabouts during the period of visual observation, with suitable resistances (for example, a variable resistance obtained by a carbon rheostat) ar-

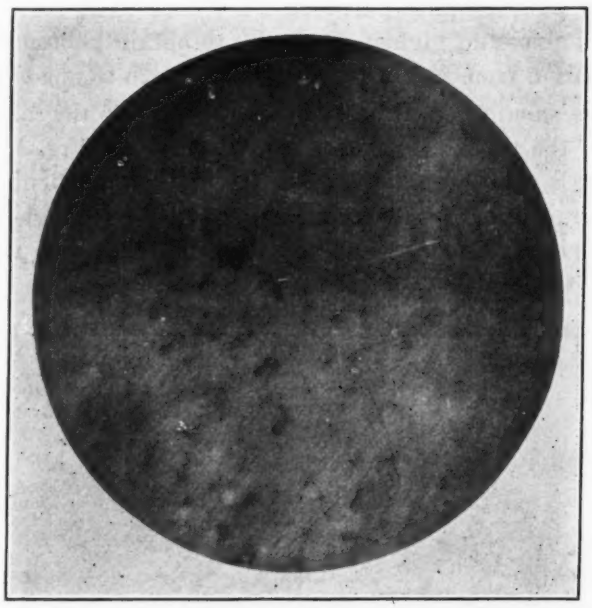

Fig. 3.—Photomicrograph of skin capillaries (X50), showing open capillaries. These can be determined numerically on the basis of number for each square millimeter of skin surface.

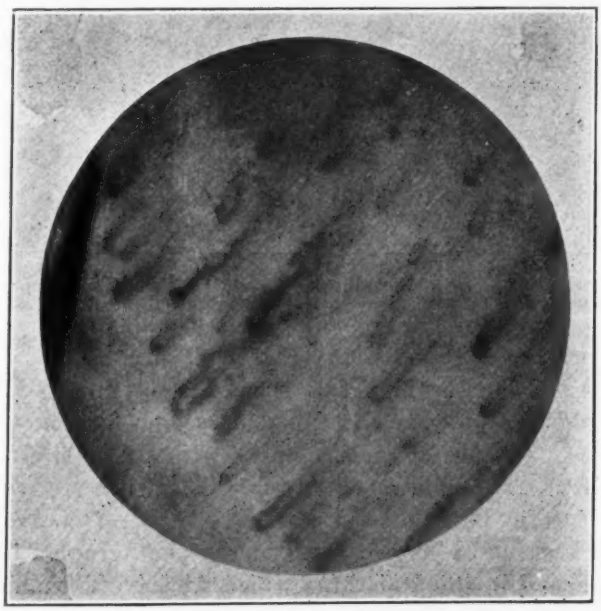

Fig. 4.—Photomicrograph. Nail-fold capillaries (X50).

ranged in parallel with the fixed resistance, so that currents of 25 to 50 amperes could be used for a few seconds when photographing. Using a total magnification of 64 (objective 16 and demonstration eyepiece 4), and

employing ordinary Eastman films, with exposure of one-tenth second, the light being concentrated upon the skin at the nail-fold by means of a suitable system of lenses (Fig. 1, e) a current of 20 to 25 amperes has proved generally satisfactory for photographic purposes. We have been able, however, using either plates or films and higher amperage, to obtain photomicrographs varying in magnification from 128 to 10 in from one-tenth to one-hundredth second.

The lantern should be angled at approximately 45 degrees with reference to the plane of the table. By means of a finger rest (Fig. 1) with its upper surface so shaped as to permit the angle of the light incident on the nail-fold to be slowly changed, the most satisfactory position and angle for photographing can be obtained through the elimination of reflections from oil surfaces and other undesirable effects.

Fig. 5.—Photomicrograph. Nail-fold capillaries (X64). Case of Raynaud's disease, showing large dilated and partially filled loops, taken during the period of cyanosis.

The construction of the camera (Fig. 1, f) needs little comment. It is made to carry a No. 118 Eastman film, since this size (80 by 105 cm.) enables one to record photographically the full microscopic field in the plane which the film occupies. The use of a roll of films with six or twelve exposures enables a series of photographic records to be taken when studying the consecutive stages in certain pathologic conditions. It is, of course, essential that the operator be assured of correct focus at the photographic film at all times. This is accomplished in the apparatus which we have assembled through the use of a Leitz eyepiece (Fig. 1, d) or similar demonstration eyepiece (magnification 4 × ordinarily used), which allows the major part of the light in the barrel of the microscope to pass through the ocular and thence to the photographic film, and at the same time reflects a portion of the light to the observing eyepiece. Before entering into the routine of taking photomicrographs, there-

fore, it is necessary to determine experimentally the position of the camera in order that the image as observed on a suitable ground glass plate laid in the plane occupied by the film, may be in as sharp focus as is the image seen in the comparison eyepiece. When this position is once determined, the clamp on the rod supporting the camera is set. No further attention to this matter is required except for an occasional check up. However, the illumination obtained by the use of large currents frequently changes the depth of focus, so that refocussing with the slow adjustment screw is required during the use of the high-powered illumination.

The photographic negatives, cut to correct size, are inserted between two thin plates of glass in the slide holder of an ordinary short focus projection apparatus. Measurements on the calibers and lengths of the arterial and venous limbs, as imaged on the screen, are made with a millimeter scale. Knowing the total magnification due to the microscope and the projection apparatus, quantitative data can be readily obtained.

Figs. 3 to 5, inclusive, are reproductions obtained from prints made from the original negatives. These selections represent average results obtained by our methods, and are taken from a group of nearly 100 cases studied.

SUMMARY

A method is presented for the successful utilization of photomicrography in the study of the smaller vessels of the skin. Complete mobilization is rendered unnecessary because of the employment of instantaneous exposures. By suitable projection methods with known magnification, quantitative measurements on the total visible loop and capillary calibers can be obtained with a small percentage of error. Permanent records are thus obtained for filing or comparative studies.

AN IMPROVED STAINING AND DRYING PLATE*

By H. C. Sweaney, M.D., Chicago, Illinois

CORPER† described an electric drying bath for staining tubercle bacillus wherein the slides were placed in a rack and allowed to steep over a layer of sand heated electrically by units composed of fine nichrome wire wound around "lavite" cores. We have used these baths continuously for a great many years, but find that in the long run they are rather expensive because the units frequently wear out and their cost is rather high. Therefore, we have been working on a model that has had for its object a reduction in price and a more general usefulness.

The heating is done by four longitudinal nichrome wire coils suspended in narrow openings in a transite base. These heating coils are far enough below the tray to prevent overheating and at the same time give ample circulation

*From the Laboratories of the Municipal Tuberculosis Sanitarium, Chicago, Ill.
Received for publication, October 28, 1924.
†Corper, H. J.: Jour. Am. Med. Assn., 1915, lxv, 420.

of air to furnish an even temperature averaging 110° C. at the level of the slide tray.

An added feature to the older apparatus is the arrangement for drying slides at a lower temperature thus obviating any chance of error in the use of blotting paper which is sometimes (too often in fact) used for that purpose.

The detailed description follows.

110 Volt A. C. or D. C. Current
Dimension over all:
Length 16" Width 8½" Height 6¼"
Weight app. 14 lb.

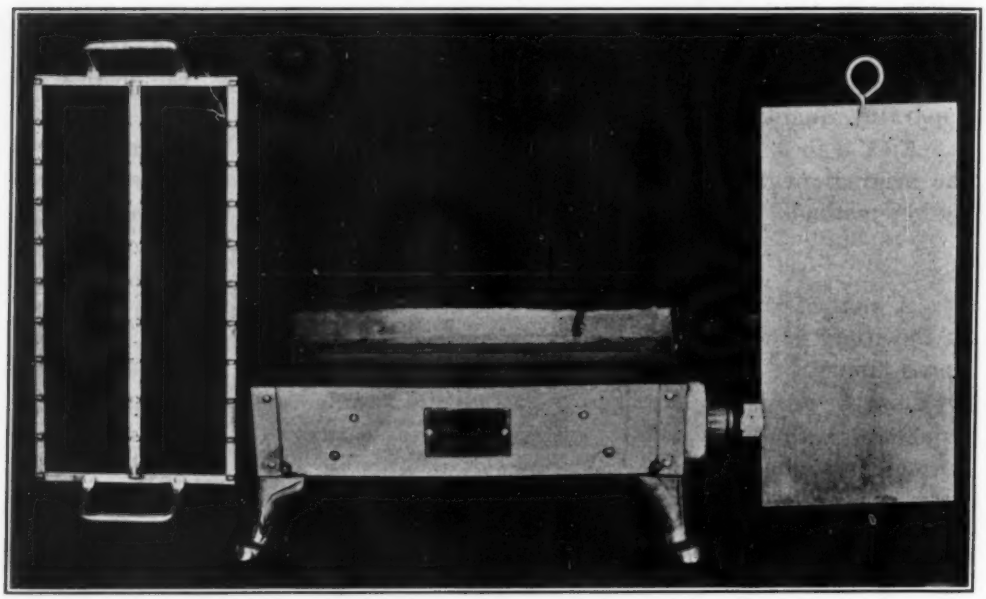

Fig. 1.

This apparatus, made entirely of aluminum, transite lined, has been designed for staining and drying bacteriologic slides. The body contains air-circulated heating coils, regulated by a three-heat switch, by means of which the desired temperature is quickly obtained and evenly maintained for any length of time. High heat is used for carbolfuchsin staining in tuberculosis work, and either medium or low heat for drying. The aluminum plate prevents injury to the elements, as it catches any staining solution, dripping from the slides. The aluminum slide rack accommodates any number of slides up to 20, and can readily be removed from the body to wash the prepared slides in the sink. A special space is provided for each slide.

A MICROMETHOD FOR THE ENUMERATION OF MICROCOLONIES*

By F. T. Briedigan and T. M. Chang, Battle Creek, Michigan

THE usual method of making a bacterial count is by the use of the counter, which is a process of division and multiplication. However, this method becomes impracticable when it comes to counting small colonies such as those of B. acidophilus, B. bulgaricus, and similar organisms which are of microscopic dimension and cannot be detected by the unaided eye. Kopeloff (by private communication) says that the usual technic of counting can be employed if the acidophilus plates are left in the incubator four or five days instead of two as commonly practiced. While the acidophilus colonies do grow larger, to some extent, with prolonged incubation, our experience seems to indicate that their size always remains microscopic, and consequently, the usual technic is hardly applicable. The difficulty is increased if the agar medium is not absolutely clear, for detritus of any sort will cause a cloudiness in the medium and will confuse with the true acidophilus colonies when examined macroscopically. Therefore, to count microcolonies with any certainty requires a definite recognition of the colonies first, in order to exclude all the pseudocolonies made of débris in the media, and for a definite recognition the microscope is indispensable. It is evident, then, that the enumeration of microcolonies can best be done under the microscope, and it is such a method, simple enough in itself but hitherto seldom used,† that we wish to describe in the following.

THE METHOD

By this method, the microscopic field is used as the counting unit corresponding to the squares of an ordinary counting plate. Since only the low power objective is used when examining microcolonies in a Petri dish, so the low power field is the basis of the microunit. The actual area of this unit will, of course, vary somewhat with the different makes of the microscope, and also with the particular ocular and tube length used, but it can be very easily calculated in each individual case according to either one of these two methods: (1) By using a stage micrometer (Hausser's), the diameter of the field can be directly read and the area calculated according to the formula ($a = \pi r^2$), or (2) if a stage micrometer is not available, a Zeiss counting chamber can be substituted. The checkered square of the chamber (Thoma ruling) is one sq. mm. and will not entirely cover the field, therefore, a fine hair must be inserted into the ocular to serve as a pointer, dividing the diameter across the field into two sections which can then be easily measured one after

*From the Bacteriologic Laboratory, Battle Creek Sanitarium, Battle Creek, Michigan. Received for publication, February 29, 1925.

†Frost has used a micromethod of counting bacterial colonies in the examination of milk with his microplate technic, but the same method has not been extensively applied where the macroplate procedure is followed.

the other with the rulings on the chamber. If the second method is used, care must be taken to see that the median line of the checkered square exactly coincides with the diameter of the field. This can be ascertained by sliding the chamber to one side with the aid of a mechanical stage until the two corners of the square touch the circumference of the field simultaneously. When this position is reached, the median line of the chamber exactly coincides with the diameter of the field. Then slide the chamber on still farther in the same direction as before until the end point of the median line touches the circumference of the field, making the first vertical line of the ruling tangent to the circumference. Thus, the distance from the end point of the median line to where the pointer touches or intersects it can be measured by the division of the counting chamber. The distance from the pointer to the other end of the diameter can be calculated in the same way, and the sum of the two gives the diameter across the field; the area can be worked out in the usual way.

In the same manner the inside area of the Petri dish is measured. The area of the dish divided by the unit gives the factor which is the number of units contained in the whole dish. With this factor the counting of microcolonies is greatly facilitated. Provided the colonies are very uniformly distributed, not less than 50 units must be counted and the average taken. A tallying register will be very useful. The latter instrument will register all the colonies counted, and this divided by the number of fields observed will give the number of colonies per unit. The number of colonies per unit times the factor equals the total number of colonies in the plate, and this times the dilution will give the number of viable organisms per cubic centimeter in the original culture.

REMARKS

All devices for the counting of bacterial colonies are bound to have more or less technical error, and it is particularly true when a count of microcolonies is attempted. This micromethod is far from being perfect, but with reasonable caution on the part of the worker, this method can be used to minimize the wide range of probable error, which would be difficult to eliminate with any other method in the counting of microcolonies. For the best result, the bacterial suspension must be thoroughly mixed with the medium, and the mixture must be poured into the plate as uniformly distributed as possible. The units selected for the counting must be uniformly scattered all over the entire plate. If 50 units are to be counted, 25 should be at and near the periphery and the other 25 at and around the center of the plate.

Bearing all the above precautions in mind, several thousands of counts have been made with this micromethod with a view to determine the minimum units that have to be counted in order to secure a somewhat constant average per unit. Table I gives the results of 2200 counts. In the first column in the table each number represents the sum of colonies found in ten fields (10 counts). In the second column each number equals the total number of colonies per 10 units plus the preceding number in the first column which is, as above stated, also the total number of colonies per another 10 units, making

the number the sum of colonies in 20 units. Likewise, each number in the third column represents the total of colonies in 30 units, etc., etc.

TABLE I

–	8								
	17	25							
	17	30	43						
	14	29	39	53					
	15	26	34	44	50				
	8	16	25	43	50	57			
	10	22	31	36	44	50	64		
	10	21	32	42	54	64	73	83	
	6	12	22	30	37	47	55	67	74
	7	20	32	41	50	60	70	82	89 99

It will be observed that the numbers in the fifth column in Table I agree with one another more closely than the numbers in any other column. Therefore, in the routine work not less than 50 units should be counted, however, the number of units to be counted is secondary in importance. For the best results, a uniform distribution of the organisms in the medium is most essential.

To facilitate work, such dilutions of the original culture should be made as will develop not more than five or ten colonies per unit. (A culture containing 300,000,000 viable organisms per cubic centimeter is best counted by this method with a dilution of 1:10,000 and checked, if desired, by a lower dilution of 1:5,000.) More than that limit will make the counting somewhat difficult, although with the aid of a tallying register it can be done nicely.

The microscope (Spencer) used by one of us (T.M.C.) has a field radius of 0.0825 cm. when a 10X ocular and the low power objective are used. This radius gives a field area of 0.021,382,515 sq. cm. The area of an ordinary Petri dish is 65.03897 sq. cm. The latter divided by the field area gives the factor which is 3041.67. This factor has been used in counting acidophilus colonies and other similar microcolonies, and favorable results have been obtained. Table II shows the result of a counting of the viable organisms per cubic centimeter from two acidophilus cultures:

TABLE II

Culture A	(dil:—1:1000)	36,436,400	per c.c.
"	(dil:—1:500)	40,080,000	" "
Culture B	(dil:—1:1000)	21,861,840	" "
"	(dil:—1:500)	17,125,108	" "

CONCLUSIONS

1. Microcolonies cannot be counted with any degree of accuracy with the usual technic under a hand magnifying lens. A micromethod is necessary for an approximate enumeration.

2. A micromethod using the microscopic field as the counting unit furnishes the worker a clear and definite recognition of the microcolonies and thus enables him to make a more reliable and comparatively more accurate count.

3. A tallying register will facilitate the work of counting greatly.

4. Provided the colonies are fairly uniformly distributed, a count of 50 fields will give a good average per unit.

SOURCES OF ERROR IN BLOOD UREA AND NITROGEN DETERMINATION*

By Elmer L. Sevringhaus, M.D., and Frances Hipple, B.S., Mendota, Wis.

IN our experience there are two sources of error in the methods for determining urea or nonprotein nitrogen in blood. Since these are frequently overlooked by technicians doing routine work, precautions are important. We have found results too high because of ammonia contained in filter paper and also in distilled water from a continuous steam heated still. The ammonia in the distilled water apparently varies, although the raw water supply is artesian from a deep stratum of sandstone. The distilled water was found to contain twice the concentration of ammonia determined for the artesian water, due to the concentrating tendency of the still. We have been using as a routine the purification of distilled water by percolation through a column of permutit, as suggested by Folin and Bell.† For this purpose we employ permutit M, supplied by the Permutit Co. at a lower price than the high grade used for most laboratory work. The only disadvantage of this process is that in the place of the traces of ammonia the water contains traces of sodium bicarbonate. Acid or alkali solutions made with this water must be standardized.

The removal of ammonia from filter paper is readily accomplished by rinsing the sheets of paper separately in ammonia-free water three times. They are dried on a pane of glass in fresh, outside air. This process was so laborious that we have searched for a brand of filter paper which could be depended on to be free from such amounts of ammonia as might interfere. For the determination of urea nitrogen by the Folin-Wu method we frequently have to use an aliquot of a 15 c.c. filtrate. The percentage error, due to contamination with ammonia, will be largest when the filtrate is small and the urea concentration low. For a 15 c.c. filtrate from a blood sample containing 10 mg. urea N per 100 c.c. a filter paper, which yields 0.0045 mg. ammonia, will cause an error of 3+ per cent. We have found only one source for filter paper which meets such a standard, using a 9 cm. paper. Eleven varieties of paper from six manufacturers were tested. Some samples showed twenty-five times this amount, which we feel is a limit of tolerance. Paper of a given variety was often variable when selected from different parts of a pack or from different shipments. The one satisfactory paper has been Durieux No. 121, a French paper distributed by the Palo Co. of New York. Numerous samples from a large shipment were found to contain 0.0048 mg. or less of N per 12.5 cm. sheet. This paper is said to be free from ammonia due to a different process from the usual one in filter paper production.

*From the Chemical Laboratory, Wisconsin Psychiatric Institute.
Received for publication, March 25, 1925.

†Folin, O., and Bell, R. D.: Applications of a New Reagent for the Separation of Ammonia, Jour. Biol. Chem., 1917, xxix, 329.

A third source of error in determining urea nitrogen is the use of too much sulphuric acid in precipitating the proteins. New technicians may do this, either due to errors in measurement or to faulty standardization of the dilute acid solution. The buffer solution prescribed by Folin and Wu is not adequate for an excess of acid.

A SIMPLE METHOD FOR THE ESTIMATION OF FREE HYDROCHLORIC ACID IN GASTRIC CONTENTS*

By Edward Hollander, M.D., New York City

THE Toepfer titration method is now generally employed for the quantitative determination of free hydrochloric acid in gastric contents. The amount of free acid calculated for 100 cubic centimeters of gastric contents is expressed as the number of cubic centimeters of tenth normal sodium hydroxide required to neutralize it. The indicator, dimethylamidoazobenzol, is very sensitive to free hydrochloric acid. It changes color when as little as 0.002 per cent of hydrochloric acid is present. It reacts with organic acids only if they are present in unusually large amounts (more than 0.2 per cent). In such concentration, organic acids are readily detected by their odor and by the Uffelmann test for lactic acid, so that their reaction with dimethylamidoazobenzol can be differentiated from that given by free hydrochloric acid.

The true acidity (or hydrogen-ion concentration) of a fluid can be determined accurately only by electrometric methods. As emphasized by Shohl and King,[1] in gastric contents, in which buffers are present, titration methods involve varying degrees of error. These depend upon the hydrogen-ion range of the indicator that is used and upon the buffer action of the fluid to be examined. However, in clinical work a high degree of accuracy is not necessary in the determination of the acidity of gastric contents. Considerable variations in acidity are found in the same individual in repeated test meals on different days. Such variations are due to uncontrollable psychic, sensory, and motor stimuli which reflexly affect the gastric secretion. Most clinicians are now agreed that the only value in estimating the free acidity of a test meal is to determine whether it is within the normal range, or whether hyperacidity, hypoacidity, or anacidity is present. The determination of absolute values for gastric acidity is without practical importance.

In fractional test meals, the exact determination of the acidity is likewise not important. Recent critical studies by Gorham,[2] Kopeloff,[3] and White[4] have shown that in accord with the physiologic experiments of Cannon, Gruntzler and others, the gastric chyme after a test meal is not a homogeneous mixture. The acid values of small aspirated fractions do not represent the acid concentration of the entire stomach contents.

*From the Medical Department, Mount Sinai Hospital, New York City.
Received for publication, December 8, 1924.

Furthermore, the routine determination of the total acidity of gastric contents gives no definite information. The total acidity is the sum, chiefly, of the free and of the combined hydrochloric acid. The amount of organic acids and acid salts is negligible in cases in which free hydrochloric acid is present. A marked increase over the normal total acidity may be due to several conditions which are grossly evident. Thus, the hydrochloric acid may be combined with blood serum in gastric bleeding, or with duodenal fluid in biliary regurgitation, or with excessive amounts of mucus of respiratory or alimentary origin.

For the reasons given, I have employed the following simple method for the determination of free hydrochloric acid, which is sufficiently accurate for practical purposes. Dimethylamidoazobenzol has a nuance of color from light yellowish pink to deep crimson, depending not so much upon the

TABLE I

COMPARISONS OF THE FREE HYDROCHLORIC ACID IN GASTRIC CONTENTS DETERMINED BY THE TOEPFER METHOD AND BY THE AUTHOR'S METHOD

CASE	TOEPFER TITRATION METHOD	TEST PAPER AND COLOR CHART
1	39 c.c. N/10 NaOH	40 c.c. N/10 NaOH
2	47 "	45 "
3	54 "	60 "
4	10 "	10 "
5	92 "	90 "
6	26 "	25 "
7	15 "	15 "
8	36 "	35 "
9	77 "	80 "
10	40 "	35 "
11	42 "	35 "
12	34 "	35 "
13	5 "	5 "
14	56 "	60 "
15	21 "	20 "

1) In the Toepfer Titration Method 10 c.c. of gastric contents filtered through gauze was used for each determination.

2) In the use of the Color Chart, when the color of the test paper lies between two colors of the chart, 5 is added to the value of the lighter color.

3) The slight variations in these comparative tests are negligible in test meal determinations.

amount of the indicator present as upon the degree of the hydrogen-ion concentration of the solution. Bibulous paper is dipped in an alcoholic solution of 0.25 per cent of dimethylamidoazobenzol and dried. This test paper when dipped into weak solutions of hydrochloric acid gives excellent shades of color. The range of color obtained is much greater than that given by congo paper. Five colors were selected representing acidities of 10, 20, 30, 40, 50 cubic centimeters of tenth normal hydrochloric acid per 100 cubic centimeters of water. These colors have been made into a chart similar to a Tallqvist hemoglobin scale.

Procedure.—One end of the indicator paper is dipped into the gastric contents and the resulting color is matched on the chart. It is important to match the paper immediately, before any evaporation occurs. The examination should be made in daylight against a white background. The degree

of the acidity is read directly. If the paper matches the deepest color (acidity of 50 or more), the gastric contents is diluted with an equal volume of water and the new reading is multiplied by two.

This method is accurate for practical purposes. Its chief advantage is its simplicity. It obviates the use of reagents, burettes, measuring glasses and other apparatus. The general practitioner, who only occasionally has to perform a gastric analysis, usually lacks the necessary materials. In an active clinic or a busy office where many test meals must be examined in a morning, the titration method becomes time consuming. Also, not infrequently, only a few cubic centimeters of gastric contents are obtained in the lumen of the stomach tube, a quantity too small for titration, but sufficient for examination by this method.

REFERENCES

[1]Shohl, Alfred, and King, John: The Colorimetric Determination of the True Acidity of Gastric Contents, Bull. Johns Hopkins Hosp., July, 1922, xxxi, 152.

[2]Gorham, F. D.: Variations of Acid Concentration in Different Portions of Gastric Chyme and Its Relation to Different Methods of Gastric Analysis, Arch. Int. Med., April, 1921, xxvii, 434.

[3]Kopeloff, Nicholas: Individual Variations as Influencing Rehfuss Fractional Method of Gastric Analysis, Jour. Am. Med. Assn., Feb. 11, 1922, lxxviii, 404.

[4]White, Franklin, W.: Simultaneous Variations in Acidity of Different Portions of Gastric Contents, Jour. Am. Med. Assn., October 28, 1922, lxxix, 1499.

TECHNICAL IMPROVEMENTS IN UREA DISTILLATION BY FOLIN-WU METHOD

By H. E. Butka, M.D., and F. E. Meisner, Los Angeles, California

SINCE the introduction of the Folin and Wu method of determination of urea in the blood, many technical improvements have been devised to simplify the technic of carrying out the test.

Our experiences with the various methods proposed have led us to develop a method which utilizes the good points of several of those already proposed together with a few minor modifications of our own. This method has been in use in our laboratories for the past year with exceptionally satisfactory results.

The accompanying photograph illustrates the instrument as used in our laboratory. We would call attention to the important points. First, the use of an ebullition tube, consisting of a short length of glass tubing sealed off at one end and inverted into the distilling mixture.

Second, the small capillary pipette which controls the rate of admission of air.

Third, the water jacket, which condenses the vapor and keeps the solution and gas coming in contact with the acid solution cool.

Fourth, capillary outlet for gases into the acid solution.

Fifth, aspiration by means of a small filter pump attached to any water outlet.

Sixth, apparatus held by a single clamp attached to water jacket.

In operation, the aspirator is turned on until bubbles appear in both tubes. Distillation proceeds until only a few drops remain in first tube.

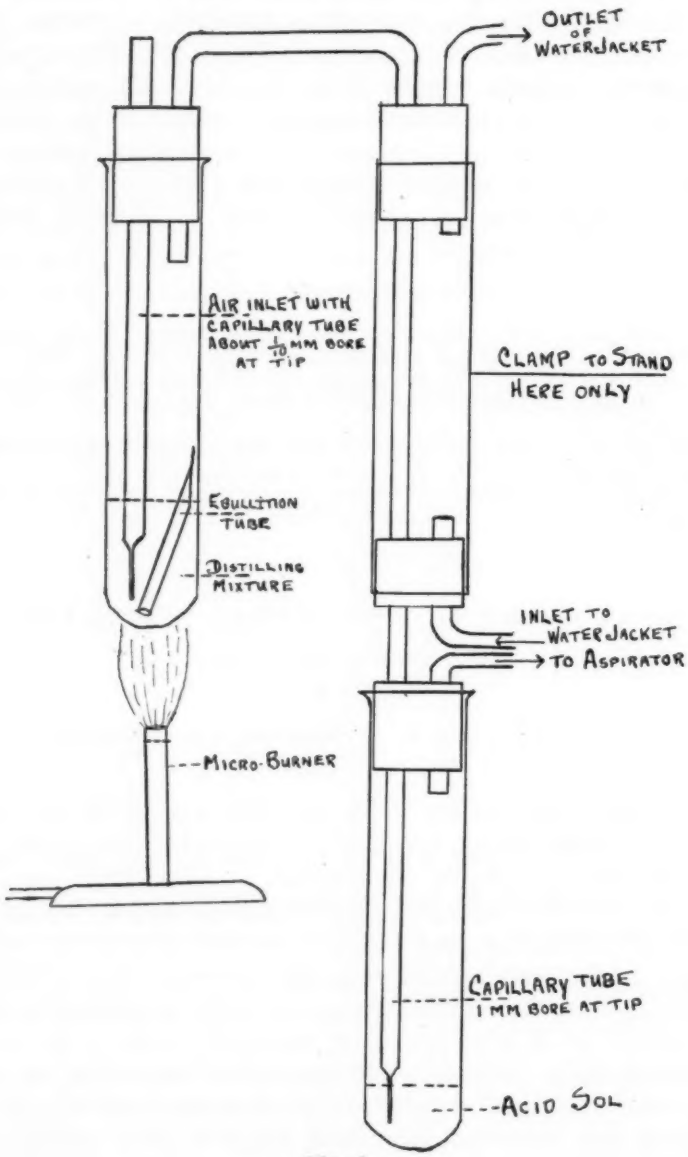
OUTLET OF WATER JACKET
AIR INLET WITH CAPILLARY TUBE ABOUT 1/10 MM BORE AT TIP
CLAMP TO STAND HERE ONLY
EBULLITION TUBE
DISTILLING MIXTURE
INLET TO WATER JACKET
TO ASPIRATOR
MICRO-BURNER
CAPILLARY TUBE 1 MM BORE AT TIP
ACID SOL

Fig. 1.

There is absolutely no back aspiration of fluid, and instrument requires no special attention during the process.

REFERENCES

[1]Folin and Wu.: A System of Blood Analysis, Jour. Biol. Chem., xxxviii, 81-112.
[2]Moore, J. W., and Jones, L.: JOUR. LAB. AND CLIN. MED., September, 1922, vii, 756.
[3]Johnson, S. Lloyd: JOUR. LAB. AND CLIN. MED., September, 1924, ix, 860., No. 12.

TRANSACTIONS

TRANSACTIONS OF THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS, FOURTH ANNUAL CONVENTION, PHILADELPHIA, MAY, 1925

THE proceedings were held in the ballroom of the Benjamin Franklin Hotel, Philadelphia, Pennsylvania, May 20 to 23, 1925.

The convention was called to order by President John A. Kolmer. The minutes of the previous meeting having been published, their reading was dispensed with. The President appointed the following members for the nominating committee: Dr. Robert A. Kilduffe, Dr. Harry J. Corper, Dr. Wm. C. MacCarty. No other business being transacted at the morning session the time was devoted to the reading of papers on the regular scientific program as follows:

The Preparation of Solutions of Dextrose for Intravenous Administration, by Dr. Ralph G. Stillman, New York City, N. Y. This paper was discussed by Dr. F. M. Huntoon and Dr. John A. Kolmer.

Utilization of Carbohydrates, by Dr. W. G. Karr, Philadelphia, Pennsylvania. (By invitation.) Discussed by Dr. Wm. G. Exton, Dr. Herman Sharlit, Dr. Frank W. Hartman, Dr. John A. Kolmer. Discussion closed by Dr. W. G. Karr.

Diagnostic Value of Spinal Fluid Sugar Content, by Dr. W. Parker Stowe, Rochester, N. Y. Discussed by Dr. John A. Kolmer, Dr. Ward Burdick, Dr. A. S. Giordano. Discussion closed by Dr. Stowe.

Alveolar CO_2 Tension and Acetone in the Expired Air in Acidosis, by Dr. Paul Roth, Battle Creek, Michigan. Discussed by Dr. A. Z. Wolodarsky, Dr. W. Parker Stowe, Dr. Frank W. Hartman. Discussion closed by Dr. Roth.

The Effect of Varied Carbohydrate Food in the Blood Sugar Concentration, by Dr. Leon Jonas, Philadelphia, Pa. (By invitation.) No discussion.

WEDNESDAY, MAY 20, 2 P. M.

President John A. Kolmer called the meeting to order and the scientific program was continued.

Aids to Urinalysis in a Large Hospital, by Dr. C. Pons and Dr. E. B. Krumbhaar, Philadelphia, Pennsylvania. (By invitation.) Discussed by Dr. Wm. G. Exton who showed a slide illustrating his method and Dr. Henry Stewart. Discussion closed by Dr. Krumbhaar.

A New System of Duplicating and Placing Laboratory Reports in the History, by Dr. Frank W. Hartman, Detroit, Michigan. Discussed by Dr. E. B. Krumbhaar. Discussion closed by Dr. Hartman.

Value of Method of Keeping Records of Tissue Examinations, by Dr. L. A. Turley, Norman, Oklahoma. Discussed by Dr. Robert A. Keilty, Dr. Ralph G. Stillman, Dr. A. H. Schade, Dr. James B. Bullitt, Dr. Frank W. Hartman, Dr. Leon S. Lippencott. Discussion closed by Dr. L. A. Turley.

Value of the Leucocyte Fragility Test in the Prognosis of Pneumonia, by Dr. C. Pons and Dr. E. P. Ward, Philadelphia, Pennsylvania. (By invitation.) Paper read by Dr. E. B. Krumbhaar. No discussion.

A Plea for a Standard Method of Determining and Reporting Hemoglobin Values, by Dr. E. Clarence Rice, Jr., Washington, D. C. Discussed by Dr. Stoner, Dr. Carl Spohr, Dr. E. B. Krumbhaar, Dr. F. E. Sondern, Dr. John A. Kolmer. Discussion closed by Dr. Rice.

A New Type of Instrument for the Estimation of Hemoglobin, by Dr. C. E. Roderick, Battle Creek, Michigan. Read by title.

The Value of the Icteric Index in Differentiating Anemia, by Dr. A. V. St. George and Dr. A. L. Brown, New York City, N. Y. Discussed by Dr. Herman Sharlit, Dr. Ralph G. Stillman, Dr. A. V. St. George. Discussion closed by Dr. A. L. Brown.

Some Useful Apparatus, by Dr. Max Shaweker, Dover, Ohio. Discussed by Dr. Frank W. Hartman. Discussion closed by Dr. Shaweker.

Clinical Classification of Cardiac Defects, by Dr. Maude E. Abbott, Philadelphia, Pennsylvania. No discussion.

The Physiology of the Circulation in Certain Congenital Cardiac Defects, by Dr. B. T. Dawson. No discussion.

THURSDAY, MAY 21, 9 A. M.

The meeting was called to order by President Kolmer and the scientific program was continued.

A Comparison of Enzyme Actions of Tumors and Normal Tissues, by Dr. K. George Falk and Helen Miller Noyes, New York City, N. Y. (By invitation.) Discussed by Dr. John A. Kolmer, Dr. Wm. G. Exton. Discussion closed by Dr. Falk.

Some Enzyme Studies with Desiccated Gonadal Tissue, by Dr. Herman Sharlit and Dr. William G. Lyle, New York City, N. Y. Discussed by Dr. K. George Falk, Dr. John A. Kolmer, Dr. H. J. Corper. Discussion closed by Dr. Sharlit.

A Double Hydrogen Electrode System for the Determination of Hydrogen-Ion Concentration, by Dr. George H. Meeker and Mr. Bernard Oser, Philadelphia, Pennsylvania. (By invitation.) No discussion.

Observations on the Dick Test, Toxin Immunization and Serum Treatment of Scarlet Fever, by Dr. John A. Murphy, Philadelphia, Pennsylvania. (By invitation.) Discussed by Dr. Kolmer, Dr. F. M. Huntoon, Dr. Herman Sharlit, Dr. E. Clarence Rice, Dr. H. A. Heise, Dr. David Bergey. Discussion closed by Dr. Murphy.

Pneumococcus Antibody Solution, by Dr. Frank M. Huntoon, Glenolden, Pennsylvania. Discussed by Dr. Wm. G. Exton, Dr. A. S. Brumbaugh, Dr. Carl Spohr, Dr. R. A. Kilduffe, Dr. A. C. Abbott, Dr. David Bergey, Dr. Robert A. Keilty. Discussion closed by Dr. Huntoon.

Autopsy Report of Two Cases of Thymic Death During Surgical Operations, by Dr. Jessie W. Fisher, Middletown, Connecticut. Read by title.

AFTERNOON SESSION, MAY 21, 2 P. M.

Meeting called to order by President Kolmer, scientific program continued.

Normal and Pathologic Basal Metabolic Rate in Obesity, by Dr. Horry M. Jones, Chicago, Illinois. No discussion.

Studies on Blood Cultures with a Special Reference to the "Massive Method," by Dr. Herbert Fox and Dr. William G. Leaman, Philadelphia, Pennsylvania. Discussed by Dr. A. I. Rubenstone, Dr. John G. Wurtz, Dr. F. M. Huntoon, Dr. Leon S. Lippencott, Dr. H. A. Heise, Dr. R. A. Kilduffe. Discussion closed by Dr. Leaman.

A Comparative Study of Liver Functional Tests, by Dr. A. I. Rubenstone and Dr. Louis Tuft, Philadelphia, Pennsylvania. Discussed by Dr. Ralph G. Stillman.

Studies on the Bacteriology of the Urine in Cooperation with Catheterization of the Ureters, by Dr. Robert A. Keilty, Danville, Pennsylvania. Discussed by Dr. A. H. Sanford, Dr. A. I. Rubenstone, Dr. Frank W. Hartman, Dr. H. J. Corper, Dr. A. H. Schade, Dr. A. S. Giordano. Discussion closed by Dr. Keilty.

A Study of Cases of Acute Leukemia and Acute Mononucleosis, by Dr. B. L. Crawford and Dr. Harold W. Jones, Philadelphia, Pennsylvania. Discussed by Dr. A. I. Rubenstone and Dr. W. Parker Stowe.

Blood Counts in Mississippi, by Dr. Leon S. Lippencott, Vicksburg, Mississippi. Discussed by Dr. James B. Bullitt, Dr. Ralph G. Stillman, Dr. W. Parker Stowe. Discussion closed by Dr. Lippencott.

Anisocytosis and Increased Red Blood Cell Volume with little or no Anemia, by Dr. Mortimer Warren, Portland, Maine. Discussed by Dr. Frank W. Hartman, Dr. H. A. Heise. Discussion closed by Dr. Warren.

THURSDAY, MAY 21, 7 P. M.

Meeting called to order by President Kolmer and scientific program continued.

Reaction After Typhoid Vaccination, by Dr. Henry J. Nichols, Washington, D. C. Discussed by Dr. John A. Kolmer, Dr. F. M. Huntoon, Dr. A. S. Brumbaugh, Dr. Robert Kilduffe, Dr. Robert A. Keilty. Discussion closed by Dr. Nichols.

The Measurement of Cloudiness in Liquids, by Dr. Wm. G. Exton, Newark, N. J. Discussed by Professor Jerome Alexander. Discussion closed by Dr. Exton.

Studies in Embalming Fluids in Relation to Necropsies, by Dr. John A. Kolmer and Dr. Fred Boerner, Philadelphia, Pennsylvania. Discussed by Professor Jerome Alexander, Mr. Papallo, Dr. A. S. Giordano, Dr. A. S. Brumbaugh, Dr. Margaret A. Miller, Dr. Case, Dr. T. H. Boughton, Dr. Ralph G. Stillman. Dr. John A. Kolmer closed the discussion.

The Specific Inflammatory Reaction of Immunized Animals (Arthus Phenomenon), by Dr. Eugene L. Opie, Philadelphia, Pennsylvania. (By invitation.) No discussion.

Methods of Staining Tubercle Bacilli, by Dr. H. J. Corper, Denver, Colorado. Discussed by Dr. Robert A. Keilty and Dr. A. H. Sanford. Discussion closed by Dr. Corper.

President Kolmer then called upon Dr. Maude E. Abbott who spoke on the Osler Memorial.

FRIDAY, MAY 22, 9 A. M.

Meeting was called to order by President Kolmer and scientific program continued.

Sedimentation Rate of Erythrocytes, by Dr. H. N. Cooper, Watertown, N. Y. Discussed by Dr. Ward Burdick, Dr. S. L. Luboff, Dr. R. A. Kilduffe, Dr. George Fussell, Dr. O. Lowy, Dr. M. A. Miller. Discussion closed by Dr. Cooper.

Standardization of Tuberculin, by Dr. Joseph D. Aronson, Philadelphia, Pennsylvania. (By invitation.) Discussed by Dr. John R. Ruchel.

Bronchial Spirochetosis, by Dr. Thomas L. Ramsey, Toledo, Ohio. Discussed by Dr. F. H. Lamb, Dr. H. J. Nichols, Dr. O. Lowy, Dr. A. H. Schade. Discussion closed by Dr. Ramsey.

Sarcinae in Psoriasis, by Miss Mary Marcus, Philadelphia, Pa. (By invitation.) Discussed by Dr. John A. Kolmer, Dr. F. L. Burnett, Dr. Martha A. Wood, Dr. H. J. Nichols. Discussion closed by Miss Marcus.

Squamous Cell Carcinoma of the Gall Bladder, by Dr. Frank W. Hartman and Dr. W. E. Johnson, Detroit, Michigan. Discussed by Dr. H. N. Cooper, Dr. A. S. Giordano. Discussion closed by Dr. Hartman.

The Technic of the Practical Application of the Pathogen Selective Cultural Method, by Dr. A. I. Rubenstone, Philadelphia, Pa. Discussed by Dr. Herman Spitz, Dr. O. Lowy, Dr. Cohen, Dr. J. J. Moore, Dr. Stella Gardner. Discussion closed by Dr. Rubenstone.

FRIDAY, MAY 22, 2 P. M.

The society was called to order by President Kolmer. The report of the Committee on Laboratory Standardization was taken up and Dr. Ruth Gilbert was asked to discuss the plan. Dr. Sondern, at the request of President Kolmer read the proposed plan and the discussion was continued by Dr. C. Y. White, (by invitation). Dr. N. P. Colwell, Secretary of the Council on Medical Education and Hospitals of the American Medical Association next discussed the proposed plan. The question was then open for general discussion.

A motion was made that the society approve the proposition as a whole and the committee be ready to present to the business meeting a specific proposal in the matter. This motion was seconded and carried. The problem was then discussed by Dr. Wm. G. Exton, Dr. Maude E. Abbott, Dr. Ruth Gilbert, Dr. K. D. Graves, Dr. H. J. Corper, Dr. J. J. Moore, Dr. R. G. Owen, Dr. Warren T. Vaughan, Dr. John G. Wurtz, Dr. L. A. Turley. The scientific program was then continued.

How Can We Best Promote the Objects Contained in Article II of Our Constitution, by Dr. Herman Spitz, Nashville, Tennessee. Discussed by Dr. Frank M. Huntoon.

Suggested Method to be Followed in Developing a Standardized Course for Medical Technicians, by Dr. Walter E. King, Detroit, Michigan. Discussed by Dr. R. A. Kilduffe, Dr. A. H. Sanford, Dr. A. S. Brumbaugh, Dr. Robert A. Keilty, Dr. F. M. Huntoon. Discussion closed by Dr. King.

FRIDAY, MAY 22, 7 P. M.

On Friday, May 22, at 7 P. M. the annual dinner was held. A sumptuous menu was served. President John A. Kolmer read his address, "Education as a Cure for Present-Day Evils in Clinical Pathology." (See page 891 of this issue.) Dr. George H. Meeker, Dean of the Graduate School of Medicine, University of Pennsylvania delivered an address on the "Functions of a Hospital." Dr. M. T. MacEachern, Director of Hospital Activities of the American College of Surgeons, who was also a guest of honor at the Rochester meeting presented a paper on the "Rôle Played By the Pathologist in Hospital Service." (See page 898 of this issue.)

SATURDAY, MAY 23, 9 A. M.

Business Session

President Kolmer.—The first order of business is the election of new members. I will call upon the Board of Censors for their report.

Dr. A. H. Schade.—The Board of Censors has gone over and approved seventy-three applications, five being for associate membership and some of these will be active members as soon as they can qualify. We have one application here which, if it is acted upon favorably by the society, establishes a precedent. The applicant is a colored physician who comes very highly qualified, vouched for by Dr. Kolmer. The Board of Censors feels that it should be brought up separately before the society as a special application.

President Kolmer.—You have heard the report of the Board of Censors, is there any discussion of this report?

Dr. Spitz.—In regard to the application from the colored physician, I am from the South. I know one colored physician in clinical pathology who is about as competent a clinical pathologist as I know. I would be the last one to vote against the applicant. According to our rules an applicant must be a graduate in medicine and a member of his local state society. If he can qualify in these respects, I would be glad to vote for him.

Dr. O. Lowy.—I move that this applicant be elected if he can qualify, and I believe he can.

President Kolmer.—I do not know whether the Board of Censors has any data. His election this morning might be unconstitutional.

Dr. Lowy.—I understood that this applicant is qualified in every way.

Dr. Spitz.—This man is not a member of his local state society. He is a member of the Kansas State Society. I am frank to tell you that under conditions existing below the Mason-Dixon line this man cannot qualify because he cannot become a member of the county society.

Dr. Sondern.—You cannot be a member of the American Medical Association without being a member of the local society.

Dr. Lowy.—Can he not qualify for associate membership? I believe we would be doing an injustice and not furthering our own cause if we kept out competent men.

President Kolmer.—That is a thought that has occurred to me. He might become an associate member. What is the pleasure of the society?

Dr. Lowy.—I move that this man be elected to associate membership.

Dr. Spitz.—I second the motion.

Dr. Sondern.—It should be stated why he was not elected to regular membership.

Dr. Lowy.—I accept the amendment.

President Kolmer.—It has been moved and seconded that we elect this candidate to associate membership, notifying him of the reasons why he could not be elected to active membership, all in favor, aye; opposed, nay; order carried.

Dr. Spitz.—I move that all the other applicants be elected to active membership as recommended by the Board of Censors. Motion seconded and carried.

A motion was made that all applicants for associate membership be elected. Motion was seconded and carried.

President Kolmer.—I would like to ask whether graduates in veterinary medicine are eligible to the society. I have in my own laboratories two doctors of veterinary medicine who

have devoted all of their professional career to the laboratory. The training that they have had in veterinary medicine has been a very good background. I would like to ask an expression of opinion as to this matter.

Dr. Spitz.—It seems to me that if they are qualified in bacteriology, they are scientific men. Section three of the constitution reads: "Associate members shall be graduates of recognized scientific institutions, who have made valuable contributions to any of the sciences relating to Clinical Pathology, or whose association with this organization will further the objects of this society. . . ." It seems to me that under that clause the two men mentioned are contributing to the welfare of clinical pathology and I should think would be eligible for associate membership in the society.

President Kolmer.—I am going to interrupt the regular order of business in order to receive the report of the Committee on Standardization of Laboratory Reagents of which Dr. Huntoon is chairman.

Dr. Huntoon.—The Committee on the Standardization of Laboratory Reagents submitted a report at the San Francisco meeting and this was published in the proceedings. It must have been satisfactory because I did not receive a single complaint. Nevertheless, the committee has been inactive for the past year because there was no further activity in that particular line; it was only by criticism from the members that we could change the standards set up. I feel that the time has arrived when the committee should be reorganized, not for establishment of standards, but for establishment of approved methods. I want to resign as chairman of this committee. I am not in a position to help with these other things. Someone who is in very active clinical pathologic practice should be the chairman of such a committee.

President Kolmer.—You have heard the report of the Committee on Standardization of Laboratory Reagents. Are there any comments?

Dr. Lowy.—I move that we adopt this report and the recommendations be accepted.

Dr. Kilduffe.—I second the motion.

The motion was carried.

President Kolmer.—We will now proceed with the regular order and ask for unfinished business.

Dr. Burdick.—At the last meeting the matter of creating the office of president-elect was brought up. It may now come up for a vote.

Dr. Spitz.—I believe that the object in that proposal last year was to follow in harmony with other organizations who have created the office of president-elect rather than first vice-president. I believe it would be a good thing to adopt. I, therefore, move that we change the clause in our constitution that we elect a president-elect instead of a first vice-president.

Dr. Burdick.—The clause as it stands submitted is to elect each year a president-elect and a vice-president in place of the present custom of electing a first vice-president and a second vice-president. This motion has been made; is the amendment acceptable to Dr. Spitz?

Dr. Spitz.—This is acceptable.

President Kolmer.—All in favor of this motion say aye, contrary nay, so ordered.

Under unfinished business I will also ask the Executive Committee to submit its report on the examination of the books.

Dr. Spitz.—The Executive Committee examined the books of the treasurer and the vouchers. We have found that the books tally with the bank. There is a statement from the bank showing a balance of $723.48 to the credit of the society. There were two items of expenditure that amounted to a considerable amount, that involved the expense of our secretary to the College of Surgeons Convention. These two trips were undertaken by our secretary with the consent of the Executive Board and we feel that the thanks of the society are due Dr. Burdick for several reasons: first, for his painstaking care in getting up papers to be read before the American College of Surgeons presenting the attitude of our society along certain lines, thereby gaining the complete cooperation of the College of Surgeons as expressed last night by Dr. M. T. MacEachern; then we owe our thanks to Dr. Burdick for the sacrifices of the valuable time that it takes, not only for such articles and papers, but for

the time he has devoted to the welfare of the society. I move that this society give a rising vote of thanks to Dr. Burdick.

President Kolmer.—You have heard the report of the auditing committee and the motion that this society, by a rising vote of thanks, extend to our secretary our appreciation of the time and valuable service he has rendered to the society.

Dr. Burdick.—I want not only to state my appreciation of your expression, but I wish to tell you that all of these efforts are shared by the so-called Denver committee, Drs. Corper and Hillkowitz, who have entered very heartily into all these activities and have given me their support to carry these things along. (Applause.)

President Kolmer.—If it is in order I would like to have a report on the financial status of the commercial exhibits in connection with the convention this year, either by the secretary or by Dr. Duck.

Dr. Burdick.—Dr. Duck feels that it is not an opportune time to make the report. He can make the report to me and we can announce it at the next convention.

President Kolmer.—We will now continue with the reports of committees. I will ask Dr. Corper to report on the Publication Committee.

The report of the Publication Committee by Dr. Philip Hillkowitz was then read by Dr. H. J. Corper.

Report of the Committee on Publication

To the Officers and Members of the American Society of Clinical Pathologists:

We submit herewith a report of the activities of the committee on publication since our last meeting at Rochester. Some time prior to the Rochester Convention, tentative arrangements had been made with the publishers of the Journal of Laboratory and Clinical Medicine to print the papers read at our scientific sessions.

During the meeting at Rochester the committee conferred with the editor of this journal and closer relations were established with the result that the Journal of Laboratory and Clinical Medicine became the official organ of the society. A substantial reduction in the subscription rate was made in favor of members of the society.

The scientific papers read at the Rochester meeting have been printed in full in successive issues of the periodical. A detailed report of the proceedings, together with the addresses of invited guests, have likewise appeared in its columns. A special department devoted to news items and official announcements of our organization has been placed at our disposal.

We desire to utilize this occasion in giving public expression of our appreciation of the courtesies shown the committee and the society by The C. V. Mosby Co., the publishers, and Warren T. Vaughan, editor, who have at all times shown a willingness to meet the requests made on them.

We are likewise grateful to them for the abundant space allowed us in the Convention Number, giving a history of the society and the program of the convention.

The question of issuing our own publication has been frequently discussed by the members of this committee, both in person at the conventions as well as by correspondence.

It cannot be gainsaid that the Journal of Laboratory and Clinical Medicine does not in all respects fulfill the ideal of an official organ devoted exclusively to clinical pathology. Objection has been made to the admixture of subject-matter which, though thoroughly scientific, yet is of interest only to a rather limited class of pharmacologists and of no particular appeal to members of our society. The lack of an Abstract Department, giving a review of current literature in the various branches of our specialty, has also been a source of complaint. We leave out of account the rather modest and backstage position our participation in the journal shows in its make-up, although most of our members, from a natural feeling of pride in their society, would like to see us occupy a more conspicuous place.

On the other hand, the arguments for publishing our own journal are met with the cold and cruel facts that it requires money as well as a considerable expenditure of time, energy and talent which, alas, in the present young stage of our organization, are lacking. All these worries are now vicariously carried by the Journal of Laboratory and Clinical Medicine.

The committee, therefore, deems it advisable for the present to continue the arrangement as before. The harmonious relations existing between the publishers of the journal and the society encourage us into the belief that such reforms as above outlined may be incorporated in the journal with benefit to its subscribers in increased usefulness and with added prestige to the American Society of Clinical Pathogolists.

PHILIP HILLKOWITZ, M.D., *Chairman.*

President Kolmer.—You have heard this report of the Publication Committee. What is your pleasure regarding it? It has been moved and seconded that this report be accepted, is there any discussion?

Dr. Sondern.—I have heard from time to time that the publishers of this journal feel that it has just begun to serve its real function and would be useful, not only to the readers, but to the owners as well. I believe that I speak correctly that they appreciate what this society has done to benefit the journal. I believe a little diplomacy between our society and those who control the journal will lead to a better arrangement. I would like to suggest that the Publication Committee use its best efforts to bring about a change in the location of the table of contents. I do not know of any of the members that understand the matter better than the committee.

President Kolmer.—Are there further comments?

Dr. Corper.—I am just informed by Dr. Exton that they have consented to make one important change that we are glad to hear about. The table of contents is going to be changed and put on one page in a better place.

President Kolmer.—I hope the Publication Committee will ask if it cannot be printed on the back page of the journal.

Dr. Exton.—We have been working towards this.

President Kolmer.—All in favor of accepting this report please say aye, contrary nay, so ordered.

I think Dr. Spitz has referred to the Executive Committee a matter relating to the method of transmitting specimens.

Dr. Spitz.—At the last meeting, at the suggestion of several of the members, first among whom was Dr. Burnett, I presented a proposition that the society issue a small leaflet in which should be printed specific instructions on how to transmit or submit specimens of various kinds to laboratories for examination. Some concrete examples of the wrong way to submit specimens to the laboratory were submitted by one of the members yesterday. This leaflet was to be issued by the society and through the various members be reissued to the general practitioners. This matter was left in the hands of the Executive Committee and referred to Dr. Stone. We regret Dr. Stone is not here. We have corresponded with him, but have not come to any conclusions about the pamphlet. I thought it would be rather presumptuous on my part to go ahead with it. Thinking of the work Dr. Sondern was doing with his committee, it occurred to me that the work might interlap, therefore, we would ask for further time so that the two committees might get together on the proposition.

President Kolmer.—Have you a motion to make?

Dr. Spitz.—I move that the Executive Committee be granted further time.

The motion was seconded.

Dr. Sondern.—The United States Postal Laws state that any specimen that contains materials that have to do with communicable diseases cannot be transported through the mails except upon receipt of a special permit from the postal authorities to receive this package. In recent applications for approval of laboratories it is stated that the applicant should, or must have a permit of this kind. Now this was put into the laws some years ago and for some time, at irregular intervals, I made application to the Post Office Department for a permit of that kind and sometimes it was acknowledged and sometimes it was not, but I never received the permit. When Dr. Hubert Work became Postmaster-General, I applied for this permit and received it in forty-eight hours. As the postal laws call for this, it might easily become one of the functions of the society. It has to do with this very committee and I think some communication should be had with the Postmaster-General's office so that it might not be so difficult to obtain these specimens that we do receive by mail. This committee might do something to help along this line.

President Kolmer.—Would that have to be a general ruling from the Postmaster-General's office?

Dr. Sondern.—It is a permit that is issued in Washington; it comes through with the mail. Many laboratories lack this permit and the material is delivered to them just the same. I think we all want to try to live up to the law.

Dr. Exton.—It would be a good thing to clear this up, I second the motion.

Dr. Cooper.—In order to obtain that approval it was first necessary to get the permit from the general post office; it took only about two weeks to get the approval.

President Kolmer.—It is a very important question. Everything may work along all right for years, then something may go wrong. I will take it upon myself to request the chairman of that committee to look into the matter.

Dr. Sondern.—Under this same law these specimens have to be packed in a specific way; these rules should be made in this leaflet as well. It is an outrage the way we sometimes receive specimens by mail. Specimens of tuberculous sputum are received in terrible condition.

President Kolmer.—It is really a matter of first rate importance. The motion, I think is understood now with the two amendments and the amendments accepted. All in favor say aye; contrary, nay; so ordered. Before proceeding with the business in order, an additional application for membership has been made and endorsed by the Board of Censors. Is it your wish that we accept this applicant? All in favor say aye; contrary, nay; so carried. We now come to the report of the committee on Laboratory Standardization, Dr. Sondern.

Dr. Sondern.—Your committee has taken into consideration every criticism and every suggestion when the subject was discussed yesterday and I think that the members of the committee are in accord with the one very good suggestion that this resolution be presented and that a good deal of discretion be allowed the committee. Therefore, I have abbreviated the report that was printed.

President Kolmer.—When you have heard this report of the Committee on Standardization of Laboratories, I will entertain a motion.

Dr. Stillman.—I move that this report be accepted and time extended to the committee. Seconded.

Dr. Lowy.—May I suggest that the doctor be licensed in the state where he is practising?

Dr. Exton.—I certainly think this committee has gone into the matter in the right way. The report is a very fine and practical one. I would like to add an amendment to it whereby the committee be empowered to confer with similar committees of the American Medical Associaiton and the American College of Surgeons and any other associations that are interseted in the subject of clinical pathology.

President Kolmer.—Is there further discussion? There are two amendments.

A voice.—I would like to know how this might apply to hospital laboratories.

Dr. Sondern.—I believe that this system of approval should apply to any laboratory. A hospital laboratory may be different from a private laboratory, but practically I know of no hospital laboratory that does not undertake the same functions as a private laboratory. You can submit a specimen of any kind to any hospital laboratory that I know of and receive a report just as from any other laboratory. That being so, I do not see why a hospital laboratory should not comply as well as the other laboratories.

Dr. Spitz.—That point is so intricate I think we ought to discuss it somewhat. Take my situation; my private laboratory is situated in an office building just one block from the hospital where I supervise the laboratory work. The technicians in each laboratory are equally competent. I divide my time between the two laboratories. Would I be required to be inspected twice and would I be required to pay two fees for the examination? I know of one clinical pathologist who lives in New York City who looks after three hospital laboratories. In that case would that man be expected to undergo three examinations in addition to his private laboratory and would he be expected to pay three fees? If I am a good pathologist in my private laboratory, why should I not be equally competent in my hospital laboratory a block away? It seems to me that one examination should suffice for both.

Dr. Lamb.—I have five laboratories. I can say further that no one of them is complete; taken all together they are complete. For instance, all the tissue work for the five hospitals is done at one hospital; no one hospital is fully equipped. Should the examination apply primarily to the laboratory or primarily to the man who is responsible?

Dr. Hartman.—This is a very important question. We are not trying to standardize only the private laboratories. I do not think that is of as much interest to us or to members. It should point more to the hospital laboratory than the private laboratory. Dr. MacEachern brought out this idea; he thought that individual laboratories scattered through the hospital were not under competent supervision. The committee apparently thinks we should not go too far in the idea of letting a man spread himself out to too many laboratories or too many hospitals.

Dr. Giordano.—I am in somewhat the same situation. I work in two hospital laboratories and I am busy enough in looking after two. As it is, it is very difficult to be in both places. There should be a limit as to how many hospitals a man can handle. One should be licensed in the state where one is practising, but so far I have not applied for a license in Indiana. There should be a time limit of one year to get a license.

Dr. Kilduffe.—While it is very intricate and very complicated we ought to avoid what Dr. Huntoon mentioned yesterday. As I understand the proposal of this endeavor to survey the laboratory situation, it was to survey it as a whole. I do not think we should go forth primarily to standardize the hospital situation or primarily to improve the private laboratory; our aims should be an approval of laboratories in general. They should include all laboratories which are engaged in the diagnosis, treatment and management of disease. It is not going to be settled in two or three minutes. It is a very difficult situation to see how laboratory activities can be spread over a tremendous territory and be correctly attended to everywhere. Every laboratory should have a certain minimum amount of equipment.

Dr. Miller.—I have a friend who is licensed in one state, but she has recently moved to another state. She is not going to stay more than a year or two and does not want to pay the reciprocity fee for that short time. In the meantime she is fully qualified to do all types of work which she is handling. At the same time, her laboratory would not be approved because she was not approved. It seems to me that some technical point might be passed over in such a case.

Dr. Exton.—It seems to me that the fact has been lost sight of that this report gives merely basic recommendations that are considered worth while in the matter of standardization. The idea of the standardization is to give good service and the committee that will have charge of this can be expected to use wisdom and discretion. I do not think there was any attempt made in this report to put hard and fast rules to our laboratories.

Dr. Sondern.—I do not think that it is possible to make any hard and fast rules. I think that when the committee functions it will have to take into consideration merits of each individual case. Where a man has charge of several laboratories I do not think that the inspection or whatever is decided on, should be considered as a separate examination; but I do think that all the laboratories with which he is connected should be inspected and then all considered one proposition. Some laboratories are extensive, others very simple. I think all this must necessarily be left to the committee that will have this matter in charge.

Dr. Spitz.—I have the utmost confidence in the integrity of this committee. I raised the question at the request of several members so that we could get a public clarification of that point. Some were a little bit dubious of the examinations. I am fully aware of the purpose of the committee and in addition to everything that has been said, I want to remind the members that this proposition is voluntary under the present system.

Dr. Martin.—It is very important that some such standardization may be had so that laboratories and men in charge that are not so qualified may be pointed out.

President Kolmer.—There is one additional point; that is the requirement that the applicant for a certificate of approval must be a graduate in medicine. What is the status of the Ph. D.?

Dr. Sondern.—The committee did not take this into consideration at all because up to the present the approval of laboratories is to be confined to the membership of this association. Now as everyone who is a member of this society must be a graduate in medicine,

we did not take into consideration the laboratories run by nonmedical graduates. I know of a number of splendid clinical pathologists that are not graduates in medicine. I would suggest that we leave that to further development and the many questions that will arise that your committee will have to deal with. They will have to come to you often for advice and counsel and recommendation and resolutions on the many, many questions that do not occur to us now. I would like to see this thing started and next year the committee will have quite an elaborate report for you.

President Kolmer.—We may now place the report for action. There have been, however, two amendments, one stating that the applicant for approval shall be licensed.

Dr. Lowy.—I think that this was discussed here yesterday, but since the committee has seen fit to reintroduce the question of licensing, they should further confine it by stating where the applicant should be qualified.

President Kolmer.—I presume that this is one of the matters that we will leave to the committee. I believe it was Dr. Exton who also offered an amendment to the motion that this committee be also authorized to confer and cooperate with the officers of other organizations engaged in similar endeavors, among which the American Medical Association, the American College of Surgeons, and the American Public Health Association were mentioned. Might we incorporate this as an amendment or may we leave it to the committee? I think we may very easily leave this to the committee. The report of this committee stands as presented. All in favor of accepting this report will please say aye; contrary, nay; so ordered.

Now I believe this brings us to new business. It has been suggested under new business that a committee be appointed to cooperate with a similar committee from the American Roentgenologic Society for the adjustment of problems common to both.

Dr. Sondern.—The association of the x-ray laboratory and the clinical pathologist came about when both of these specialties were young. I remember well, years ago when radiology first came in, I thought that it was going to be one of the functions of the clinical pathologist. As a matter of fact, I succeeded when I was in Berlin in actually bringing the first x-ray machine to America. We as clinical pathologists are no longer associated more closely with the roentgenologist than we are with any others and I do not think that in this modern day there is any need for any association with roentgenologists to further the purposes of this society. I would like to express my disapproval of complicating our functions in any such manner as has been proposed.

Dr. Exton.—I move it be tabled.

President Kolmer.—Is there any further discussion? I will take the suggestion that has been made and place the matter on the table. It has also been suggested that a committee be appointed to consider the matter of standardization of laboratory procedures. I think this is a splendid suggestion.

Dr. Burdick.—That was suggested to me by a member and I told him I would call your attention to it.

Dr. Spitz.—As I suggested in my paper, the third object in Article II is to establish uniform standards in laboratory work. I do not think that uniform standards are desirable. If we say that a certain method is better than others we remove the incentive to further research according to the second object of Article II, and I suggest the term optional standards. We may have more than one procedure that may give equal results. We can arrive at the same result by two or three different methods. I think the term optional standards should be used and two or three methods that are desirable, available and accurate be allowed to be used. Personally I prefer the Ziehl-Neelsen method for tuberculosis. I think I can find tubercle bacilli by my method just as quickly. These other methods are equally as good. It should be left to the individual as to what method he prefers.

Dr. Exton.—I would, therefore, suggest that the Committee of Reagents as it is going to be reorganized, deal with the whole question of laboratory methods, including reagents, and I think Dr. Spitz's point was very well taken. Approved methods are what we have in mind. That would cover this optional idea. This committee's functions should be broad enough to include apparatus. Some day the society might publish a book having all of its approved methods. I, therefore, make a motion to the effect that the committee be organized to deal with this matter and report at the next meeting. The motion was seconded.

President Kolmer.—It is now open for discussion and further comments. It is passing over a big burden to the committee to shoulder this enterprise. I believe it is a matter of first rate importance, particularly if the committee can get the cooperation of the society. This is done by the chemical societies with considerable profit to its members. The committee will have a very important task.

Dr. Hartman.—I agree with the chairman that this is an extremely important subject, and getting more and more so. We at least ought to have these procedures listed and I am coming to the opinion that the report ought to include something about the method as to how this procedure should be accomplished. We should have a list of what we approve and let people know of what we approve.

President Kolmer.—I will put the motion now to vote. All in favor say aye; contrary, nay; so ordered.

The American Society of Clinical Pathologists already have in operation a bureau for aiding institutions in securing the services of a pathologist. This can be developed into a department of great service. My special purpose is to ask for an expression of opinion of a bureau for the registration of laboratory technicians; a bureau to which a technician may apply; a bureau to which we may refer. I think this can be developed into a bureau that would be of considerable service. It is now open for any expression of opinion.

Dr. Burdick.—With reference to the machinery already set up, at the suggestion of Dr. Kilduffe some six months ago we started this Service Bureau and at that time I sent a postal card to some nine hundred Class A hospitals of one hundred beds and over, apprising them of this new department. I have about twenty requests from hospitals and possibly as many from clinical pathologists and I have reason to think that we have probably placed one or two clinical pathologists in positions through this bureau. It is a thing which should be brought to your attention and should be developed.

Dr. Sondern.—I move that this matter of registration of technicians be referred to the Executive Committee with power. Motion was seconded.

Dr. Hartman.—The American Society of Bacteriologists has such a bureau and I have tried once or twice to use this and found it quite unsatisfactory. These people are always out of a job and there is a good reason for their being out of a job. If this society takes up this matter they certainly should take measures to know the qualifications of people registering with them.

Dr. Spitz.—May I ask an expression of opinion as to whether the society should derive any monetary benefit from this bureau or is it to be a free service? Of course, if the Executive Committee recommends it, it will be understood that each applicant is competent.

President Kolmer.—Is there further discussion? If not, I will put the question to vote. All in favor that this be referred to the Executive Committee with power say aye; contrary, nay; so ordered.

Dr. Spitz.—I brought up a matter in my paper that is very close to my heart and I believe close to the hearts of other members of the society. Dr. Sondern in his report, mentioned several matters that do not really come under the project of approval of laboratories in the technical sense, but come under the matter of the moral methods of the individual. I might refer specifically to the matter of splitting of fees. I do not see where this would really come under the provision of the standardization. We have in our constitution the clause that the code of ethics of the American Medical Association is our code. I have gone over this code of ethics; I have read it time and again. There are a number of factors in that code that are not applicable to this society. It seems to me that there are many things that should be incorporated in a code of ethics that will cover our relationship, not only with one another, but with the physicians at large, with our technical help and association with hospital connections and also with the public. The time has arrived when this society should have its own code of ethics which can clearly state just exactly what our attitude is on many of these problems. With that thought in view I made the proposal. I now move that you appoint a committee to seriously consider this matter of adopting and endorsing a special code of ethics to cover this society. The motion was seconded.

Dr. Sondern.—I move an amendment to this motion now that the proposition be handed to our committee on revision of by-laws.

Dr. Kilduffe.—I second the amendment.

President Kolmer.—Is there further discussion? It has been moved and seconded that we refer to our Committee on Constitution and By-Laws the matter of drawing up a code of ethics for the American Society of Clinical Pathologists. All in favor say aye; contrary, nay; so ordered.

Dr. Moore.—There was some criticism that our society was not cooperating with the American Medical Association. Dr. Colwell would like to have this society act as the inspectors of laboratories for the standardization of laboratories by the American Medical Association. You know the American Medical Association is composed of units. As the Association has a Hospital Committee which acts on the hospitals that are approved, I would like to have a member of this society in each state if possible become a member of this Hospital Committee; and that member could act with him through the committee in standardizing the laboratories. He feels that every man in this organization is capable of being inspector. He also wishes a minimum standard of laboratories to be drawn up by this organization to be given him to use in standardization of laboratories. We thought it well to make a little closer cooperation, but I think that Dr. Colwell was elected a member of this organization today, so that there will be no trouble in getting cooperation. The next thing we have to do is control entirely the Committee of the American College of Surgeons. There was a point that was brought up yesterday in regard to the free Wassermanns being done by the state. In Illinois we have an Illinois Laboratory Association recently started. The question was brought up at our Tuesday meeting this week how we could stop free Wassermanns from being done by the state where the patients were being charged by the physician for the service. The state was doing it for two purposes: to control the contagion and to give a lower service to the patient. Neither purpose has been fulfilled. I found out that in Illinois, instead of controlling the syphilitic contagion they simply have the state examine the Wassermanns by numbers. I asked the director of the laboratory why they were using this method. He said that they wanted to know how many positive and how many negative Wassermanns there were in the state. I am sure that every laboratory in the state would be very glad to cooperate with the Health Department and send them a report each year or each month of the Wassermanns done in that particular laboratory. A motion was passed that every Wassermann done by the state have the name of the patient and address and that this be sent back to the doctor. We should have a notice sent to the patient that this Wassermann was done at the expense of the state and is public property and that this was done free and there should be no charge on the part of the physician.

President Kolmer.—You brought up three or four subjects. In order to clarify the matter and expedite our consideration, submit them to us in the form of a motion.

Dr. Moore.—I would move that we would cooperate with the secretary of the American Medical Association as closely as we can in his inspection and standardization of laboratories.

Dr. Stillman.—I second the motion.

Dr. Spitz.—That point is already covered by a motion by Dr. Exton that we appoint a committee to confer with the different organizations. I believe the committee already has this function in view.

Dr. Exton.—I think under the circumstances it would be a good plan to put it in the form of a motion and put it to a vote so that they have a better standing when they come to these conventions. The motion is that the committee which is in power to proceed with the matter of standardization be empowered and instructed to confer and cooperate with the authorities of the American Medical Association, the American College of Surgeons and the American Public Health Association with the idea of joining together to better the conditions among laboratories of the country.

President Kolmer.—You have heard the motion, is there any discussion? If not all in favor will please say aye; contrary, nay; so ordered.

Dr. Moore.—I would move then that this organization draw up some form of resolutions to be presented to the state laboratories in regard to the unfair performance of Wassermanns.

President Kolmer.—Is this motion seconded? The motion was seconded.

President Kolmer.—It is now open for discussion.

Dr. Spitz.—I would personally, from a selfish standpoint, like to see the public health laboratories discontinue doing Wassermanns. This is a public health problem. We, of course, quite approve of the various methods being used by the Venereal Disease Boards throughout the country. In my city the public health laboratories are doing Wassermanns and I question whether the evil is sufficient to overcome the good that they are doing. I know there are a lot of patients that are having Wassermanns done by the state that could afford to pay a laboratory fee and there are certain physicians who charge patients for Wassermanns and send them to the state laboratory. I question whether we ought to attempt anything at this time in addition to the other things that we are trying to accomplish which I think are far more important. I believe it would be advisable to let that particular subject alone.

Dr. Graves.—I have a strong feeling that the laboratory, as such, is being crowded out of existence by the public health laboratories. We feel that the performance of Wassermanns is not a public health measure. No ethical laboratory worker would refuse to do a free Wassermann on someone who could not afford to pay for it. We are cutting down the supply of workers by making the field less attractive to them. In my state there has not been a man who has gone into this branch of work for many years. The state is taking over the entire thing. I am ready to offer an amendment that we go on record as being opposed to the State Health and City Health Departments doing Wassermanns on patients other than those coming under their direct control. I would like to offer a suggestion that we appoint one or two counselors in each state to act as a Board of Recourse for this society.

Dr. Lowy.—The free Wassermann is of very little importance; the actual danger to clinical pathologists is that eventually, unless that evil is stopped, they will go very much further than just doing free Wassermanns. Until this organization was formed the clinical pathologists had absolutely no standing. In our city they are giving away hundreds of tons of coal to those who cannot afford to buy it, but if I were to try to get some of this free coal I could not get it; yet they are doing free Wassermanns for people who can well afford to pay for them. The State Board of Health in Rhode Island is attempting to do free blood chemistries. They are infringing upon our right to live. One of our reasons for being in existence is to make a good fair living. We will not be able to do this unless we are willing to stop all this.

Dr. Rhamy.—I think this is the biggest subject that has come before this society. I am from Indiana and our Board of Health not only does every kind of laboratory test, but it advertises what it is doing. The head of the laboratory is not a pathologist at all; he is a health officer and the work is all done by technicians. This man goes to county societies and asks physicians to send their laboratory work to the state. The free Wassermann is the worst of all these. I believe that unless this evil is combated by this society the future of the clinical pathologist will be very dark.

Dr. Stier.—I just want to endorse what the last two speakers have said. In coming through Salt Lake City, I visited the State Laboratory where everything is being done, from tissues on down. It is in charge of a man not fully trained in pathology and not a graduate of medicine. I think it is up to us to take some action to try to curb this state of affairs.

Dr. Owen.—We have the same problem to face in Michigan. I would like to make an amendment that a committee be appointed to take up and investigate this whole question and report to us at some future time.

Dr. Sondern.—I would like to second the amendment of Dr. Owen. This association cannot afford to do anything that is contrary to the interests of public health. I agree with everything that has been said. I would ask the committee that they be most stringent in their investigations.

Dr. Keilty.—I would like to say a word or two on the thought that Dr. Sondern has just raised regarding any action that we may take which may seem to be against public health. These public health societies were organized for the benefit of public health. The

point has been raised several times as to the manner of handling this problem. In Pennsylvania there is a state committee and the head of the laboratory of the department is also a member. It seems to me that if such an organization of committees of the state medical societies were operating through each one of the states they could handle the matter, keep the abuse down, and help along on the good side of it. We ought to be very careful in opposing anything that is for public health.

Dr. Vaughan.—I personally entertain a doubt whether we are justified in taking the stand that because one can afford to pay a fee for a Wassermann one should go to a private laboratory. The man who can afford to pay it pays larger taxes than the man who cannot afford to pay. I think that what should be objected to is the doing of so-called "routine" Wassermanns in our state laboratories. Wherever there is a suspicion of syphilis or outspoken or treated syphilis, I doubt whether we would gain much in the way of cooperation if we try to put any objection up to the health authorities. I cannot see the justice of the practice of many clinicians in sending routine Wassermanns done in the same way as routine hemocytologic or urine examinations to the public health departments.

Dr. Schade.—This question has been thrashed out in Ohio for the last four years. Recently a tissue was sent to the city laboratory in one of the cities in Ohio; the man in charge is a bacteriologist and knows nothing about pathology, so he referred it to another man who had just arrived in the city with very little experience. He made a diagnosis of carcinoma. Fortunately for the patient perhaps, the man sent him to a surgeon. The slides were asked for in this case and were examined and nothing malignant was found. The man said that the slide that showed malignancy was lost. There are a great many evils in this and how to approach them is a question. We, in Ohio, have been on the job for four years; it is a hard proposition. We have taken it up through the local, city, and state societies and we have not arrived anywhere yet.

Dr. Lamb.—From the amount of discussion on the subject something should be done about it. I have had a concrete idea for some time; that is, to point out how much the laboratories are costing the state, then to propose that this service can become self-sustaining and that in order to do so, Wassermanns and tissue examinations should be made only for those who cannot afford to pay for them and that in the case of the Wassermann, for instance, the patient himself sign a statement saying that he is unable to pay the usual fee. The patient could have the service of the state laboratory but he would pay for it. I think there is a thought there that will appeal to the state laboratories. I think that if it is brought before them that there are hundreds and thousands of dollars of possible revenue in the state laboratories they would see the point pretty quickly.

Dr. Lowy.—I would like to bring out another point. That is the absolutely unfair competition. We subscribe to a code of ethics which says that we cannot advertise. The state department goes further than that; it sends out letters paid for by our taxes and competes with us in an unfair way. All that it wants is to do a lot of work so that the director can make a big showing. Now it is perfectly all right for this association not to do anything which would be subversive of public health, but permitting public health authorities to do free work that belongs to clinical pathologists is not fair. We must be treated fairly by our brothers in medicine.

President Kolmer.—I am lost in the maze of amendments. I think I recollect the original motion, I am sure it was seconded, that the matter be referred to a special committee to be appointed by the President to investigate this subject and to report back at our next convention. I am sure that all of you will agree that it is a very important motion. I hope that we will appoint to this committee individuals who will fully realize the importance of the work laid upon them. All in favor say aye; contrary, nay; so ordered.

Dr. Burnett.—I have a matter that I would like to take up. Lately several printed forms of health examinations have come out in some of the medical journals. The laboratory part of the examination was very inadequate. It seems to me that this matter might be referred to the Executive Committee within this organization to take up this matter with the other committees and perhaps make up a minimum requirement for adequate laboratory examinations. I move that a committee in this organization be empowered to take up

the matter of the inadequate laboratory examination in the health examination with other committees that are making up forms for such examinations. Motion seconded.

President Kolmer.—This motion is now open for discussion. As I understand it we would place upon our Executive Committee the duty during the ensuing year of drawing up what that body would consider a proper series of laboratory examinations to be recommended in conjunction with health examinations. All in favor please say aye, contrary, nay; so ordered. I am ready to receive the report of the nominating committee.

Dr. Schade.—For President for the ensuing year, Dr. Frederick E. Sondern; President-Elect, Dr. William G. Exton; Vice-President, Dr. F. L. Burnett; Secretary-Treasurer, Dr. Ward Burdick; Member of Executive Committee, Dr. Philip Hillkowitz; Member of Board of Censors, Dr. George Ives.

President Kolmer.—You have heard this report, is there any discussion?

Dr. Spitz.—Mr. Chairman, I move that the report be accepted and all of the nominations submitted by the nominating committee be elected. Motion seconded.

President Kolmer.—It has been moved and seconded that this society by a standing vote elect the officers proposed by our nominating committee; all in favor kindly rise. It has been unanimously carried.

The next subject is the selection of the next place of meeting.

Dr. Sondern.—As it has been customary for this association to meet at least near by and approximately at the time of the meeting of the American Medical Association, I would move that the determination of the place and time of the meeting be left to the Executive Committee with power. Motion seconded.

President Kolmer.—You have heard this motion, is there any discussion? If not, all in favor say aye; contrary, nay; so ordered. I believe this terminates the business of the morning. I am not going to be denied the pleasure of ushering the new President into office; that will be reserved for the afternoon session.

SATURDAY, MAY 23, 2 P. M.

The meeting was called to order by President John A. Kolmer and the scientific program was continued. The papers were all read and then discussed as a whole.

The Clinical Significance of Anticomplementary Serum, and Spinal Fluids in the Wassermann Reaction (Kolmer Modification), by Dr. A. H. Sanford, Rochester, Minnesota.

A Standardized Wassermann Report, by Dr. A. J. Casselman, Camden, New Jersey.

The Present Status of the Kolmer Complement-Fixation Test for Syphilis as Determined by a Critical Comparison with Numerous Other Methods, by Dr. Robert A. Kilduffe, Atlantic City, New Jersey.

Comparison of Results with Kolmer Wassermann Method and Kahn Precipitation Test, by Dr. Robert G. Owen, Detroit, Michigan.

Kahn's Precipitation Reaction as Compared to Kolmer's Complement-Fixation Test, by Dr. A. S. Giordano, South Bend, Indiana.

A Clinical Study of the Kolmer and Kahn Reactions in Syphilis, by Dr. R. L. Kelly, Louisville, Kentucky. (By invitation.)

The Meinecke Reaction as Compared with the Wassermann in One Thousand Specimens of Blood Sera, by Dr. A. M. P. Saunders, Dunning, Illinois. Paper read by Dr. Herman Spitz.

President John A. Kolmer with a few well chosen words then ushered Dr. Frederick E. Sondern into office.

Dr. Sondern.—I appreciate fully this honor. I am grateful to you for your confidence. I will do my best to merit what you have done.

You have put before your officers and representatives two very difficult tasks. The more difficult of the two is this consideration of what is proper for Public Health, State, County and City Laboratories to do in the way of work and what is not proper. This is one of the most difficult problems I know of, but I am going to try, with the help of the committee, to solve it as best we can. I do not think we can solve it entirely, but we are going to try to bring you our best explanation of it. I am serious in this matter and I want to assure you that your officers are going to do all they can to carry out your wishes

in this long interval. I thank you again for this honor and as I said before, I am going to do my very best.

The scientific program was continued and the papers that had been read were opened for discussion. Discussed by Dr. Martin, Dr. A. J. Casselman, Dr. Henry Stewart, Dr. Herman Spitz, Dr. H. J. Nichols, Dr. Robert A. Keilty, Dr. Frank W. Hartman, Dr. R. A. Kilduffe, Dr. John A. Kolmer.

There being some time left, Dr. B. S. Parks read his paper, The Bactericidal Action of Whole Blood as Determined by the Heist-Lacy Method. (By invitation.)

The meeting was then adjourned.

The Journal of Laboratory and Clinical Medicine

VOL. X ST. LOUIS, MO., AUGUST, 1925 No. 11

Editor-in-Chief: WARREN T. VAUGHAN, M.D.
Richmond, Va.

ASSOCIATE EDITORS

DENNIS E. JACKSON, M.D. - - CINCINNATI
PAUL G. WOOLLEY, M.D. - - DETROIT
J. J. R. MACLEOD, M.B. - - - TORONTO
ROY G. PEARCE, M.D. - - AKRON, OHIO
W. C. MACCARTY, M.D. - ROCHESTER, MINN.
GERALD B. WEBB, M.D. - COLORADO SPRINGS
VICTOR C. MYERS, PH.D. - - IOWA CITY
MARSH PITZMAN, M.D. - - - ST. LOUIS
RUSSELL L. HADEN - - ROSEDALE, KAS.
JOHN A. KOLMER, M.D. - PHILADELPHIA
ROBERT A. KILDUFFE, M.D. - ATLANTIC CITY, N. J.
A. PARKER HITCHENS, M.D. - - MANILA, P. I.

Official Organ of the American Society of Clinical Pathologists

Contents of this Journal Copyright, 1925, by The C. V. Mosby Company—All Rights Reserved
Entered at the Post Office at St. Louis, Mo., as Second-Class Matter

EDITORIALS

The Philadelphia Meeting

IN a perusal of the transactions of the American Society of Clinical Pathologists which appear elsewhere in this issue, one will gain an excellent insight into the character of the agenda. While the purposes of the society are known to any who care to read the constitution and by-laws or the brochures which have been published by the society, the real test of any organization's value is in its accomplishments in and out of business session.

During its relatively short existence the A. S. C. P. has already to its credit: the evolution of an idea; the gathering to its standard of representative men and leaders in clinical pathologic thought; the acquiring of full recognition from older medical organizations; and the inception of appropriate steps towards the realization of the idea,—the ideals for which the society was organized.

We need not discuss here the high caliber of the scientific contributions, since they will appear later on their own merits, but the journal takes real pleasure at this time in congratulating the society on its accomplishments, and on its successful annual convention.

—*W. T. V.*

Cholesterolemia and the Complement-Fixation Test for Syphilis

DESPITE the enormous amount of study which has been devoted to the complement-fixation test in syphilis we are still without definite knowledge as to the exact mechanism responsible for the production of a positive reaction.

"While lipoidal extracts, as well as normal and luetic serums, may separately absorb or fix a small amount of complement, a mixture of a suitable extract and a suitable (syphilitic) serum is capable of fixing large amounts of complement"[1]—and that is all that is known, so far, of the mechanism concerned in the production of the reaction in syphilis.

It is highly probable that the true explanation of the reaction may lie in the field of colloid chemistry and that changes in the surface tension and alterations in the character and dispersion of colloidal particles in the serum, complement, and antigen are factors of decided importance.

That lipoids are of extreme importance was first brought out by the introduction of cholesterolized antigens, concerning the reliability and specificity of which there has been much discussion. From the fact that the addition of cholesterol to an antigen led to an increase in the number of positive reactions obtained with it, to a speculation as to the effects of an increase of cholesterol in the fluids examined was a natural step.

Among the early observers to credit an increased cholesterol content as a factor capable of producing a postive reaction was Pighini[2] who, finding a large amount of cholesterol in 88 per cent of Wassermann positive spinal fluids, concluded that the cholesterol content was an important factor, if not the main cause, of the positive reactions.

Henes[3] not only believes that the interpretation of the significance of a positive reaction must be directly governed by the cholesterol content of the specimen—of less significance when the cholesterol is high than when it is low or diminished—but also maintains that a high cholesterol content may be the direct cause of a positive reaction.

This assertion, if true, would be of vital importance to both serologist and clinician as depriving the complement-fixation test in syphilis of all significance unless a cholesterol determination is simultaneously performed, and unless the specimen examined showed a normal or diminished amount.

The statement, however, has not been allowed to pass unchallenged.

Cholesterol forms a part of every mixed diet and, while the power of the body to synthesize cholesterol from cholesterol-free substances is still a matter of doubt, that the blood cholesterol content can be deliberately increased by the ingestion of foodstuffs rich in this substance has been definitely shown.

If, therefore, a mere increase in the blood cholesterol is a factor of im-

portance in the production of a positive complement-fixation test, it should be easily demonstrable by artificially producing a hypercholesterolemia by feeding experiments and sera so obtained should exhibit a positive complement-fixation test.

Such a series of experiments has been reported by Craig and Williams.[4]

These investigators chose for their experiments the rabbit because, first, this animal is known to give a positive complement-fixation reaction after experimental infection with the Spirocheta pallida, and, second, because it has been demonstrated that the blood cholesterol of these animals can be definitely increased by feeding cholesterol.

To a series of ten rabbits known to give consistently negative complement-fixation reactions (over a period of ten days preceding the experiment), cholesterol was fed in large amounts.

Prior to the experiment cholesterol values were shown to range from 85.5 to 133 milligrams per 100 centimeters of blood. Similiar determinations during and after the termination of the feeding experiments gave values showing an increase of from 284.3 per cent to 680 per cent above normal values. Complement-fixation tests during these periods of extreme hypercholesterolemia were consistently negative.

It may be thought that such experiments, while of interest, are only hypothetically comparable to similar conditions in the human being. It is permissible, therefore, to speculate as to what would have been the results under similar conditions of hypercholesterolemia in human serum and the query finds an answer in the work of Thalhimer and Hogan.[5]

The plan pursued by these investigators was to make a quantitative complement-fixation test after the method described by Kolmer and thus determine with great exactitude the complement fixing value and then, after adding to the positive serum a known negative serum having a high cholesterol content, to repeat the test on the assumption that, if cholesterol affects the results of complement-fixation tests, then increasing the cholesterol content of the tested serum should also increase the strength of the reaction.

The hypercholesterolemic sera were obtained from cases of pregnancy, jaundice, diabetes, and nephritis and the cholesterol content ranged from 250 milligrams per cent to 833 milligrams per 100 centimeters of serum, the normal value in the human being ranging from 160 to 180 milligrams per cent.

The negative sera which, it is to be noted, were negative in spite of their high cholesterol content, were all tested for the presence of natural antisheep hemolysins which, if present, were removed by absorption.

The results of the experiment were entirely uniform: The hypercholesterolemic sera were negative in the first place and when added to known positive sera in a quantitative test not only failed to increase the strength of the reaction but this on the contrary, was decreased owing to the dilution of the positive sera by the addition of the negative sera. These observers, therefore, concluded that hypercholesterolemia was not a factor in the production of a positive complement-fixation reaction in syphilis and cannot thus be the cause of false or nonspecific positive reactions.

The pathologic factors concerned in the production of hypercholesterolemia are not well understood. While it is true that some of the methods recognized as at times giving false positive reactions with a majority of the methods in common use, such as diabetes and pregnancy, are not infrequently associated with hypercholesterolemia—though this is not constant—it is equally true that in other conditions, such as pneumonia during the febrile stage in which false positive reactions are not infrequently obtained, the blood cholesterol is markedly decreased.

Syphilis, however, is the one disease in which positive complement-fixation reactions are consistently found. If hypercholesterolemia is responsible even in part for the production of the positive reaction, then hypercholesterolemia should be consistently associated with syphilis.

An investigation along these lines is reported by McFarland[6] who found that of a series of serologically positive syphilitics only 6.8 per cent showed a high cholesterol value, 75.9 per cent having a "medium" content, and 17.2 per cent having low values. He concludes, therefore, that there is no relation between the cholesterol content of the serum and the occurrence of a positive reaction.

Of interest, also, is the work of Levinson, Landenberger, and Howell[7] who determined the cholesterol content of 53 positive spinal fluids, examining 25 by both qualitative and quantitative methods, and 28 by qualitative methods alone. In none could a reading be made by the Bloor method nor was there any inhibition of hemolysis by the saponin test.

In only three fluids—one luetic and meningitic combined, and two from general paresis—was it possible to demonstrate the presence of cholesterol.

It appears fair to conclude, therefore, that the presence of cholesterol has no bearing upon the production of positive complement-fixation reactions in luetic spinal fluids, and that pathologic or experimental increase of cholesterol in either the blood or spinal fluid has no influence upon the production of a positive complement-fixation reaction in syphilis and no bearing upon the interpretation of the reaction.

REFERENCES

[1]Kolmer, J. A.: Infection, Immunity, and Specific Therapy, Philadelphia, ed. 2, 1920, W. B. Saunders and Co., p. 429.

[2]Pighini: Ueber den cholest. der lumbalhussigkeit ein Gusteskrankeiten, Hoppe-Seyler's Ztschr. f. physiol. Chem., 1909, lxi, 508. Cited by Levinson, Landenberger, and Howell, Note 7.

[3]Henes, E.: Cholesterolemia and the Wassermann Reaction, Jour. Am. Med. Assn., 1915, lxiv, 1969; ibid, Am. Jour. Syph., 1920, iv, 1.

[4]Craig, C. F., and Williams, W. C.: Experimental Observations upon the Effect of Cholesterolemia on the Results of the Wassermann Test, Am. Jour. Syph., July, 1921, v, 3.

[5]Thalhimer, W., and Hogan, B. M.: The Inability of Serum with a High Cholesterol Content to Increase the Strength of the Wassermann Reaction, Arch. f. Dermat. u. Syph., April, 1923, vii, 4.

[6]McFarland, A. R.: Studies in the Blood Cholesterol in Syphilis, Arch. f. Dermat. u. Syph., July, 1922, vi, 39.

[7]Levinson, A., Landenberger, A., and Howell, K.: Cholesterol in the Cerebrospinal Fluid, Am. Jour. Syph., April, 1921, clxi, 589.

—*R. A. K.*

Color Scales and Complement-Fixation Reactions

THE occurrence of a positive complement-fixation reaction is evidenced and its degree determined by the absence of hemolysis in the corpuscle suspension added as an indicator.

Because of the quantitatively adjusted proportions of the complement, antigen, and serum doses on the one hand, and the amboceptor and corpuscle doses on the other, it is obvious that various readings are possible from complete hemolysis to its complete inhibition, and to record these gradations the so-called Citron scale has long been in use and is familiar to everyone.

As concerned with the development of uniformity in the performance and reading of the test, attention has been again directed toward refinements in the use of the Citron scale as a means of determining with greater or less exactitude the intensity of the reactions obtained.

In a recent communication, Gilbert, Kelly, and Moore[1] report the result of their studies in this direction culminating in the construction of a new and carefully prepared indicator standard or color scale which they suggest as tending toward uniformity in reporting the results of the test.

From time to time in the literature of the complement-fixation test as applied to the diagnosis of syphilis, various methods of preparing the corpuscle suspension have been proposed, from attempts to standardize the suspension by actually counting the cells, to attempts at standardization by the estimation of the hemoglobin; none, however, has attained any great vogue nor, in the last analysis, are they a necessary or even a logical concomitant of a "standard" technic, nor do they achieve more than an apparent exactitude.

One of the underlying purposes of attempts to develop a "standard" technic is to render possible the comparison of the results obtained by different workers on the same or different sera or of the same worker on serum obtained from the same individual at different times.

That this can be brought about with entire satisfaction by attempts to standardize the corpuscle suspension is open to question.

In the first place, while it is quite possible that the individual worker may more closely approximate his own results from day to day by the use of a color scale, it by no means follows that his results may be quantitatively compared with those of other workers, even though they are using the same scale, unless they are using an identical technic in the performance of the test.

Inasmuch as the occurrence of hemolysis or its inhibition is directly related to and influenced by the quantitative relations existing between the various reagents, no exactitude in the preparation of reading scales will render possible the exact comparison of results by two methods in which the other quantitative factors are markedly at variance. The sensitivity of the antigen; the period and temperature of the primary incubation; the serum and complement dose must all be considered and unless all are comparable, the tests may only be compared in a gross way as to whether they are negative or positive—

and even here, there may be a perceptible or even pronounced disparity when one technic is much more delicate than another.

Reading scales may well serve to achieve a little more exact differentiation between a plus-four and a plus-three in the same laboratory with the same technic but cannot per se serve to impart a strictly quantitative factor, for methods utilizing a single or even a double dose of serum are essentially qualitative in character and serve merely to establish the presence or absence of reacting substances.

That it is important to have some means of estimating the strength of reactions is admitted and there can be little debate as to the necessity of a quantitative factor in any "standard" technic; but as long as we remain uninformed as to the underlying mechanism of the complement-fixation reaction; as long as we are ignorant as to the substances producing the positive reaction, just so long will attempts to measure the strength of the reaction be clouded with difficulty and more or less empirical.

There is, it is true, a difference in the intensity of a plus-four and a plus-one reaction read with or without a scale, but the difference is quantitative only in the sense of a quantitative difference between a "large amount" and a "trace" of albumin in a qualitative test in the urine; a gross difference—no more and no less.

Serologists and clinicians are coming to accord as to the necessity for a quantitative factor; the question is concerning the best method whereby it may be obtained.

In the present state of our knowledge, as has been discussed elsewhere,[2] a quantitative factor appears possible in only one of three ways:

1. By determining how great a number of antigen units a given quantity of tested serum can utilize in the presence of a set dose of complement.

2. By determining how great a number of complement units a given quantity of serum can fix in the presence of a set dose of antigen.

3. By determining how small an amount of serum is capable of producing any degree of fixation, however minor, in the presence of a set dose of complement and antigen.

Of these the last has the most advantages and the least disadvantages and is altogether the most feasible.

It is apparent that a serum which is capable of giving fixation of plus-two degree in an amount of 0.0025 c.c. contains more reagin than one which gives fixation only in 0.05 c.c. even though the degree of fixation be plus-four in the latter amount. If emphasis is placed—as it may be—not upon the *degree* of fixation but upon the *quantity of serum* with which it is obtained, the reaction becomes more strictly quantitative and, where different workers are using even varying technics but all are using graded doses of serum, their results become comparable in degree if the same or comparable doses of serum are tested, even though the plus-three of one worker be the plus-four of another.

If both obtain fixation with 0.0025 c.c. of tested serum both have obtained a reaction the strength of which may be, at least, compared; though refinements of technic may influence appreciably the degree of inhibition of hemolysis or even its occurrence in a stated serum dose.

It would appear that uniformity of results as related to the strength of the reaction obtained is more likely to be attained through the use of graded amounts of tested serum which may be more easily prepared than reading scales, if not, perhaps, more accurately, and which present, moreover, a logical as well as a ready means of measuring that upon which the strength of the reaction depends—the reagin content of the serum.

REFERENCES

[1]Gilbert R. Kelly, M. F., and Moore, A. C.: The Reading of Complement-Fixation Tests by the Citron Scale as Compared with a Method Using a Color Scale, JOUR. LAB. AND CLIN. MED., April, 1925, x, 552.

[2]Kilduffe, R. A.: Concerning the Necessity for a Quantitative Factor in a Standard Complement-Fixation Test for Syphilis and the Methods Whereby it may be Obtained, Ann. Clin. Med., January, 1925, iii, 481.

—*R. A. K.*

Notice

The continually increasing cost of production in the printing business makes it necessary that we raise the subscription price of the JOURNAL OF LABORATORY AND CLINICAL MEDICINE.

Beginning with the October issue, the subscription price will be increased $1.50 per year. The size of the Journal will be increased possibly two hundred pages during the year. This increase is necessary in order that we can accommodate the large amount of worth-while material that is being continually offered to the Journal and that should be published.

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

---

*Concealed Tuberculosis**

THE author takes the stand that "that tired feeling" in individuals in whom no physical cause can be found and which is usually attributed to neurasthenia, is often due to latent active tuberculosis. In detecting the source of the infection, he employs the subcutaneous tuberculin test using as his criterion a febrile reaction. He requires no focal reaction since he maintains that often in these cases the infection is so well concealed that no focal reaction can be recognized by present methods of examination. The cutaneous tuberculin reaction is not as trustworthy. Pathologic proof of tuberculous infection is not easy because these patients rarely die within the period of observation.

The treatment recommended is the usual "preventorium" treatment.

The book is essentially a brief for the recognition of latent active tuberculosis without localizing foci as a cause for various neurasthenic symptoms grouped under the popular term "The Tired Sickness," and for the more general use of the subcutaneous tuberculin test in the recognition of these cases.

Principles of Biochemistry†

THE second edition of Robertson's *Principles of Biochemistry* contains the usual proportion of alterations and additions, necessitated by advances in our knowledge of the subject. The sections dealing with the regulation of the neutrality of the blood and with metabolism in diabetes have been considerably expanded. The chapter on growth has been greatly altered and a new chapter has been added on the relation of growth to diet. New sections deal with the racemized proteins, the functions of the nucleic acids and on the environment as a factor in the evolution of the higher animals.

After considering the inorganic foodstuffs and the significance of foodstuffs in general, the author discusses the chemistry of the carbohydrates, the hydroaromatic derivatives, fats, proteins and amino acids, nucleic acids and the nitrogenous bases. He next takes up the hydrolyzing enzymes and finally the digestion and assimilation of various foodstuffs.

The second portion of the book deals with the properties of protoplasm. This is chiefly a consideration of the colloidal chemistry of the body.

---

*Concealed Tuberculosis or the Tired Sickness. By George Douglas Head, B.S., M.D. Pp. 137. Cloth. Price $2.00. P. Blakiston's Son & Co., Philadelphia, 1924.

†Principles of Biochemistry. By T. Brailsford Robertson, Ph.D., D.Sc. Second edition, revised. Illustrated with 57 engravings. Cloth. Pp. 796. Price $8.50. Lea & Febiger, N. Y., 1924.

Part Three is of unusual interest in its mode of presentation. In it the author deals with the chemical correlation of the tissues. First, he discusses from the biochemical viewpoint the vehicles of correlation, the blood and lymph. Second, he describes the chemical correlation of respiratory activity, the chemical regulation of the circulatory system, the chemical correlation of the processes of digestion, of the organs of generation and the chemical regulation of metabolism.

Part Four deals with the chemical processes which underlie and accompany life's phenomena. This includes the intermediate metabolism of the various foodstuffs, the energy transformations in living organisms, fertilization, early development, growth and the relation of growth to diet, memory and sleep.

Section Five deals with the waste products and Section Six with the animal body as a machine, or the energy balance of the organism.

---

*Serum Diagnosis of Syphilis by Precipitation**

THE great interest which has developed within the last two years in the various precipitation tests for syphilis, particularly the Kahn test, has rendered it highly desirable that an authoritative discussion of the reaction in all of its phases be presented in one volume.

The author presents, in all necessary detail, historical notes, the governing principles of precipitation with their application, and a detailed description of his test as applied in practice.

Although there is distinct difference of opinion regarding the ultimate reliability of precipitation tests as opposed to the standard Wassermann reaction, the fact that according to the author's figures, his reaction as now employed, agrees with the standard technic in 97 per cent of tests, demonstrates that it has a distinct place in the serology of syphilis. In many localities indeed, it is being relied upon almost entirely as a diagnostic procedure, and a clear understanding of its mechanism, as brought out in this book is therefore highly desirable.

The argument for the test would perhaps have been slightly more complete had the author taken up in a special section, a discussion of those criticisms that have appeared in the literature.

Compend of Genito-Urinary Diseases and Syphilis†

AS a compend, this is very good. All genitourinary conditions except perhaps the exceedingly rare, are briefly discussed and the more generally accepted views are presented. Recently developed procedures and methods of treatment such as tryparsamide in neurosyphilis, mercurochrome, etc., are incorporated. Good illustrations are included.

---

*Serum Diagnosis of Syphilis by Precipitation. Governing Principles, Procedure, and Clinical Application of the Kahn Precipitation Test. By. R. L. Kahn, M.S., D.Sc. Michigan Dept. of Health, Pp. 237. Cloth. Price $3.00. Williams & Wilkins Co., Baltimore, 1925.

†A Compend of Genito-Urinary Diseases and Syphilis. By Charles S. Hirsch, M.D. Fourth edition, revised. 44 illustrations. Pp. 337. Cloth. Price $2.00 P. Blakiston's Son & Co., Philadelphia, 1925.

The American Society of Clinical Pathologists

Dr. Frederick E. Sondern, New York
President

Dr. Wm. G. Exton, Newark, N. J.
President-Elect

Dr. F. L. Burnett, Boston, Mass.
Vice-President

Dr. Ward Burdick, Denver, Colorado
Sec'y-Treas.

Executive Committee:
Dr. Herman Spitz, Nashville, Tenn.
Dr. A. H. Sanford, Rochester, Minn.
Dr. Philip Hillkowitz, Denver, Colo.

Board of Censors:
Dr. A. H. Schade, Toledo, Ohio
Dr. Ernst A. Victors, San Francisco, Cal.
Dr. George Ives, St. Louis, Mo.

Officers of the Society

At the fourth annual meeting held in Philadelphia, in May, 1925, the following officers were elected to serve the Society during the year 1925-1926:

Officers

Dr. Frederick E. Sondern, President, New York, N. Y.
Dr. Wm. G. Exton, President-Elect, Newark, New Jersey.
Dr. F. L. Burnett, Vice-President, Boston, Massachusetts.
Dr. Ward Burdick, Secretary-Treasurer, Denver, Colorado.

Executive Committee

Dr. Herman Spitz, Nashville, Tennessee; Dr. A. H. Sanford, Rochester, Minnesota; Dr. Philip Hillkowitz, Denver, Colorado.

Board of Censors

Dr. A. H. Schade, Toledo, Ohio; Dr. Ernst A. Victors, San Francisco, California; Dr. George Ives, St. Louis, Missouri.